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MANUAL OF MICROBIOLOGIC MONITORING OF LABORATORY ANIMALS

DEPARTMENT OF HEALTH AND HUMAN SERVICES
Public Health Service
National Institutes of Health

MANUAL OF MICROBIOLOGIC MONITORING OF LABORATORY ANIMALS

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Preface

In May 1980 the governments of Japan and the United States of America established a joint program for Cooperation in Research and Development in Science and Technology. Because those responsible for this activity recognized that the use of research animals would be vital to the development of many biomedical activities envisioned in this venture, they included a laboratory animal science project. Within this project a number of collaborative activities have taken place, including the fostering of information exchange.

An area in which complementary information was available in both countries was the microbiologic monitoring of laboratory animals. It became evident that the available information would be more valuable if brought together from diverse sources in both countries and organized into a manual which could be used by laboratory animal scientists everywhere. Meetings were held both in Japan and the United States to plan such a manual.

It was decided that the first edition of the manual would be concerned only with mice and rats and would have two parts. The first, an introduction, would provide in-depth perspectives and guidelines on monitoring from the viewpoint of producers and users of animals; it would also include a list of infectious agents of mice and rats considered to be potential pathogens or agents that interfere with research. The second part—the main body of the manual—was to consist of presentations of selected agents very likely to be monitored either routinely or under special circumstances. In keeping with manual style, pertinent categorical information would be briefly presented, including a description of the agent, characterization of the infection, methods of control, preferred and alternative methods of testing, and directions for the preparation of test reagents. Loose-leaf format was chosen to facilitate subsequent insertion of information on additional agents and the revision of data as the field progresses. It also became evident that the value of this publication could be extended if authors would be invited to contribute from institutions and countries worldwide. An initial step has been taken in this first edition with the participation of Dr. Frank Fenner of Australia.

We believe that this volume represents a successful conclusion of the initial plans for the development of such a manual and envision that it will become a perpetually valuable document, with new contributions to be added later and with information to be periodically updated in this rapidly moving field.

We who are assigned counterpart responsibility for the initiation of this project wish to express our gratitude to those many contributors who have made it possible to satisfy our highest expectations. We are particularly appreciative of the efforts of Dr. A.M. Allen, who has had the primary responsibility for coordinating this activity.

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Introduction

The purpose of this manual is to provide a framework of information needed to establish microbiologic monitoring programs for different kinds of animal colonies. Comments are offered here to deal with questions that continually arise concerning the extent of monitoring deemed necessary and how to sample animal colonies for monitoring purposes. The following treatments of these subjects provide no final answers, but it is hoped they will air the subjects and help those in decisionmaking positions to select suitable approaches.

Perspective and Philosophy of Microbiologic Monitoring of Laboratory Animals

An increasing awareness and demand for laboratory animals of high quality and definition is evident today. Researchers are striving to reduce variability by using a "defined" animal, and government regulations require that the laboratory animals not be infected with microbial agents which would interfere with drug testing and cancer studies.

Though research workers historically have been concerned about the quality of laboratory animals, there was little hope for upgrading or expecting better quality until the late 1950's or early 1960's when cesarean derivation and production of mice and rats under barrier conditions became a reality. The literature of the first half of this century contains many accounts of spontaneous or naturally occurring diseases of laboratory animals which were studied and recorded because of interference with the intended research. During the past two decades we have seen an increased use of cesarean-derived, barrier-maintained mice and rats to study the naturally occurring disease conditions of conventionally raised rats and mice. The utilization of mice and rats free of pathogens (except for vertically transmitted agents such as tumor viruses) is rapidly becoming commonplace. This does not mean that once free of pathogens, a production colony remains so indefinitely. Numerous so-called "breaks" have been recorded. Furthermore, the maintenance of pathogen-free mice and rats during the conduct of research requires the use of special isolation facilities and procedures designed to prevent the introduction and spread of unwanted microbial agents in the animals. To a great extent, the state of the art has been such that producers have been able to provide cleaner mice and rats than some researchers were prepared to use properly.

The pendulum swings both ways, however. The increasing visibility assigned to the defined animal and the increasing awareness of the researcher regarding the effect of infectious agents, including some not normally thought of as pathogens, have catalyzed the need to develop the means to monitor the animals more precisely. Thus, there is need for further research on the naturally occurring diseases, including the development of more sensitive and specific diagnostic techniques.

Many people with diverse objectives have an interest in defining the laboratory animal, of which microbiologic monitoring is only a part. To assist our communication and understanding, the definition of a few commonly used terms is appropriate.

Quality control. This is a broad and inclusive term which embraces all measures taken to define, maintain, and monitor those factors that might result in animal variability. It includes, but is not limited to, environment (e.g., temperature, humidity, lighting, air filtration, noise), husbandry (e.g., caging, cleaning, feeding, watering), nutrition, toxic substance detection, genetic profile, and health assessment. It is the sum of the activity which permits a statement regarding the definition of the animal.

Health Assessment. This is also a broad term which includes all efforts to evaluate or appraise the state of health (or freedom from disease) of animals. It is an integral part of a good quality control program. Health assessment of a single animal or group of animals might include information regarding reproductive performance, morbidity and mortality data, gross and histologic pathology, detection of endo- and ectoparasites, isolation of actual or potential pathogenic microbial agents (bacteria, rickettsia, fungi, or viruses), and various serologic procedures to detect previous exposure to specific or serologically related agents. It is the total activity which describes the health of an animal (or group of animals) at one point in time.

Health assurance. Though this term implies desirable elements of confidence and security, it is a poor term for health assessment because it suggests a guarantee, a term from which biologists tend to shy away.

Microbiologic monitoring. The term monitoring implies to check or test for quality, to keep track of, to regulate or control. Microbiologic monitoring of laboratory animals is the process of repeated testing for evidence of infection with specific agents at specified time intervals. It might be considered a process for testing whether the system (e.g., cesarean derivation, environmental control, husbandry) is functioning properly. Such testing should permit a statement regarding the *expected quality* of animals coming from a highly regulated environment such as a closed strict barrier-maintained colony or isolator-maintained animals.

From the foregoing discussion of terms it should be clear that *microbiologic monitoring is but one aspect of health assessment, which is but one aspect of the quality control program designed to produce optimally defined animals for the researcher.*

For the purpose of initiating this manual, mice and rats only are considered since they are the species most commonly used in biomedical research, and production of mice and rats with a defined gastrointestinal microflora has been highly successful. Production of mice and rats in isolators as germfree animals following cesarean derivation is too expensive to fulfill most needs of biomedical research. The successful compromise has been achieved by providing a known gastrointestinal microflora and maintaining production under barrier conditions. Though we wish to make a distinction between barrier-produced animals and conventionally produced animals, we recognize the inherent variability of colony management practices of various producers. Terms such as clean conventional and modified barrier are encountered, and one cannot say categorically that mice and rats produced by conventional practices (air not filtered; food not pasteurized or autoclaved; water, bedding, and cages not sterilized; and no special precautions by attending personnel to prevent spread of agents) are ubiquitously infected with a number of pathogens while those produced under barrier conditions are not infected. Nevertheless, conventionally raised mice and rats are more likely to be infected with various pathogens than the cesarean-derived barrier-maintained counterpart. Furthermore, it is considered more prudent to monitor for the presence of an unwanted agent in a population where the agent is not expected to be present (barrier) than in a population where the probability of the agent being present is greatly increased (conventional).

For this reason, and because monitoring is time-consuming and can be expensive, we advocate its application mainly for cesarean-derived, barrier-maintained mouse or rat colonies. Exceptions might be the requirement of a research protocol or the gathering of prevalence data.

One of the most debated questions regarding microbiologic monitoring of laboratory animals is "which agents should one test for?" The most important consideration, and indeed the final word, rests with the needs of the investigator. Animals should be produced or procured that are free from experience with certain agents that would interfere with the investigator's research. It is necessary therefore that a dialogue be established between the producer and the investigator. Though monitoring procedures are not a substitute for controls in the experiment, the investigator should receive information from the producer attesting to the required quality of the animals. During the production of laboratory animals, the responsibility for microbiologic monitoring should rest primarily with the producer. Quality control and health assessment, including microbiologic monitoring, are best accomplished at the production level. It is here that base populations can be defined for purposes of establishing statistically significant sampling procedures; assessing reproductive performance, morbidity and mortality data, and necropsy results; isolating microbial agents and analyzing the results of serological procedures; and performing an overall assessment of the health of the animals.

As a microbiologic monitoring procedure, the routine repeated testing of significant numbers of mice and rats from strict barrier-maintained production colonies *for all known or potential pathogens is probably not justifiable*; it can be expensive, and experience indicates it is redundant. Microbiologic monitoring of potable water and milk for human consumption as a quality control procedure, for example, does not include tests for the innumerable pathogens of man. Rather, the test is for evidence of fecal contamination, evidence of a "break" in the processing system. Such an analogy may have merit in deciding which test(s)

to perform to detect a "break" in the system of cesarean-derivation and strict barrier production of mice and rats. Though the human being is considered the weakest link in animal production procedures, the major threat is not the direct transmission of pathogens from man to the animals (though this can happen) but rather failure to prevent the introduction of murine pathogens by fomite transmission to the animals. It seems appropriate, therefore, to frequently monitor the barrier colony for one or more of the *most prevalent and communicable murine agents* for which sensitive and specific tests are available to detect a "break" in the system. Likely candidates might be Sendai virus (mice and rats), mouse hepatitis virus (mice), and rat coronavirus sialodacryoadenitis virus (rats). The finding of positive results should alert management to investigate immediately the nature of the break and the probable source of the contamination. Development of a thorough in-depth profile for purposes of "definition" and "health assessment" of the rodents can be performed at less frequent intervals as experience with the production facility dictates. Additionally, tests for certain agents (e.g., ectromelia, LCM, and hemorrhagic fever viruses) may be required when shipping rodents between laboratories or countries.

The foregoing is intended to address microbiologic monitoring aspects of the health assessment of mice and rats during the production phase primarily. The production of defined mice and rats free of recognized pathogens (with the exception of certain tumor viruses) has been demonstrated repeatedly. However, when the animals leave the confine of the vigorously controlled environment of the production facility, the risk of contamination increases. To profit from the effort of producing pathogen-free animals, they must be protected from possible contamination during transit, at receipt, and during experimentation. The animals must be maintained in a controlled environment free from exposure to contamination and microbiologically monitored to ascertain this status during experimentation. The principles and philosophy of microbiologic monitoring discussed previously are applicable to the experimental phase of animal use as well as to the production phases. The *needs of the investigator* deserve reiteration when deciding which agents should be selected. The needs of biomedical research requiring use of animals are too numerous and varied to permit the compilation of suggested lists of agents for which to test. This selection is best accomplished through a close working relationship between the researcher and those persons responsible for procuring, maintaining and monitoring the animals. The maintenance of mice and rats in a pathogen-free state during the conduct of research is an attainable goal.

Considerations in the Sampling Procedure for Microbiologic Monitoring of Laboratory Animals

Microbiologic monitoring of a representative sample from a given population is the most appropriate means of determining whether the given population is presently infected (isolation or antigen detection procedures) or has been infected (serological procedures) with a given agent. Monitoring each animal in the population is time-consuming, expensive and, if the determination requires killing the animal, it is self-defeating.

Significance of the results of the sampling procedure depends upon the application of statistical methods. To detect a *single* case of disease in a population of 100 or more animals at the 95 percent confidence level, the sample size = log 0.05/log N, where N = the percentage of normal animals (ILAR News, 1976). To increase the confidence limits to the 99 percent level, substitute log 0.01 for log 0.05 in the numerator. It is essential to recognize that the application of this formula depends upon an assumption that (a) *the sample is representative of the base population* (a random sample) and (b) *the expected prevalence rate for the specific disease in a comparable population of 100 or more animals is known*. The importance of these considerations underscores the contention that the primary responsibility for microbiologic monitoring should rest with the animal production facility.

The character of the animal production facility may vary between animal producers (e.g, room size, cage size, population within each cage, use of filter caps on cages, air filtration, and numerous husbandry practices). Such variables may affect the random sample. The base population should be defined in each instance: Is that population contained in an isolator, in a single production room, in a stock (issue) room, in the entire production facility, or in a single shipment? Most of the prevalence data available today was obtained from survey of conventionally maintained mouse and rat colonies. Extrapolation of this information to the expected prevalence rate of infection by a specific agent in a cesarean-derived, barrier-maintained production colony may not be valid. It is essential to recognize that differences between species of animal, strains of animal species, pathogens, strains of pathogens, sensitivity of testing procedures,

stratification of the population (e.g., exbreeders vs. weanlings), husbandry practices, and numerous other variables may affect the expected prevalence rate of infection in a population (colony). Failure to define such variables does not preclude the possibility of sampling a population. However, failure to recognize the importance of such variables may reduce or invalidate the significance of the results of the sampling procedures.

The complexity of the effects of the numerous variables mentioned above is such that a more practical approach to the sampling procedure is often taken. With numerous assumptions and a sample size of ten (10), one might be satisfied with the detection of at least one case of infection with a given pathogen when the prevalence rate reaches 30 percent in the colony. For the more highly communicable infections, this seems quite adequate. Familiarity with the colony resulting from microbiologic monitoring and other health assessment procedures should provide the information needed to regulate the sample size.

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Reference

Long-term holding of laboratory rodents. ILAR News (Institute of Laboratory Animal Resources, National Research Council) 1976; 19 (4): 22-23.

List of Microbial Agents of Mice and Rats

This list is intended to be neither inclusive nor exclusive, but represents a compilation of known or suspected pathogens or agents that may interfere with biomedical research.

VIRUSES	Agent occurs naturally in Mouse	Rat
Ectromelia	+	–
Hantaan and related viruses	+ *(wild)*	+
K	+	–
Lactic dehydrogenase (LDH)	+	–
Lymphocytic choriomeningitis (LCM)	+	–
Minute virus of mice (MVM)	+	–
Mouse adenovirus (MAd)	+	?
Mouse cytomegalovirus (MCMV)	+	–
Mouse encephalomyelitis (Theiler's, GD VII)	+	–
Mouse hepatitis (MHV)	+	–
Mouse mammary tumor viruses (MMTV, Type B retroviruses)	+	–
Mouse rotavirus (EDIM)	+	–
Mouse sarcoma & leukemia viruses (Type C retroviruses)	+	–
Mouse thymic virus	+	–
Pneumonia virus of mice (PVM)	+	+
Polyomavirus	+	–
Rat corona/sialodacryoadenitis (RCV/SDAV)	–	+
Rat cytomegalovirus (RCMV)	–	+
Rat parvovirus (KRV, H-1)	–	+
Ratpox	–	+
Reovirus 3	+	?
Sendai	+	+

BACTERIA	Agent occurs naturally in Mouse	Rat
Actinobacillus spp	+	+
Bordetella bronchiseptica	+	+
Cilia associated respiratory bacillus	+	+
Citrobacter freundii, atypical	+	–
Clostridium spp	+	+
Corynebacterium kutscheri	+	+
Escherichia coli	+	+
Klebsiella spp	+	+
Leptospira spp	+	+
Mycobacterium avium-intracellulare	+	–
Mycobacterium lepraemurium	+	–
Pasteurella spp	+	+
Pseudomonas aeruginosa	+	+
Salmonella spp	+	+
"Spirillum minus" (no reference strain exists)	–	+
Staphylococcus aureus	+	+
Streptobacillus moniliformis	+	+
Streptococcus pneumoniae	?	+
Streptococcus spp	+	+
Tyzzer's agent (Bacillus piliformis)	+	+
Yersinia pseudotuberculosis	+	+

MYCOPLASMA	Agent occurs naturally in Mouse	Rat
Mycoplasma arthritidis	+	+
Mycoplasma collis	+	+
Mycoplasma muris	+	–
Mycoplasma neurolyticum	+	–
Mycoplasma pulmonis	+	+

RICKETTSIA & CHLAMYDIA	Mouse	Rat
Eperythrozoon coccoides	+	–
Grahmella spp	–	+
Hemobartonella muris	+	+
Rickettsia akari	+	+
Chlamydia psittaci	+	–
Chlamydia trachomatis	+	–

FUNGI*	Agent occurs naturally in Mouse	Rat
Aspergillus spp	+	+
Candida tropicalis	+	–
Cryptococcus neoformans	+	–
Histoplasma capsulatum	+	–
Phycomycetes spp	–	+
Trichophyton mentagrophytes	+	+

*Except for *T. mentagrophytes*, this list comprises agents rarely encountered in laboratory or free-living mice or rats.

HELMINTHS**	Mouse	Rat
Aspicularis tetraptera	+	+
Capillaria hepatica	+	+ (rare)
Heterakis spumosa	–	+
Hymenolepsis diminuta	+	+
Hymenolepsis nana	+	+
Syphacia muris	+	+
Syphacia obvelata	+ (rare)	+
Taenia taeniaformis	+	+ (rare)
Trichinella spiralis	+	+
Trichosomoides crassicauda	–	+
Trichuris muris	+	+ (rare)

**For a more complete list of helminths occurring in laboratory and free-living rodents worldwide, see "Parasites of Laboratory Animals" by Robert J. Flynn, 1973, Iowa State University Press.

PROTOZOA	Agent occurs naturally in Mouse	Rat
Balantidium coli	–	+
Cryptosporidium muris	+	–
Cryptosporidium parvum	+	–
Eimeria falciformis	+	–
Encephalitozoon cuniculi	+	+
Entamoeba muris	+	+
Giardia muris	+	+
Hepatozoon muris	–	+
Klossiella muris	+	–
Pneumocystis carinii	+	+
Sarcocystis muris	+	+
Spironucleus muris	+	+
Toxoplasma gondii	+	+
Tritrichomonas muris	+	+
Trypanosoma lewisi	–	+

ARTHROPODS

	Agent occurs naturally in	
	Mouse	Rat
Laelaps echidinus	+	+
Myocoptes musculinus	+	–
Myobia musculi	+	–
Notoedres muris	–	+
Ornithonyssus bacoti	+	+
Polyplax serrata	+	+
Polyplax spinulosa	–	+
Psorergates simplex	+	–
Radfordia affinis	+	–
Radfordia ensifera	–	+
Trichoecius romboutsi	+	–

*Descriptions of Selected Agents and Methods for Diagnosis and Monitoring

*The looseleaf method of preparing this manual will permit revision and updating of these descriptions periodically as new information becomes available. Likewise, it is planned that descriptions of other agents of importance to monitoring programs will be added.

I. Viruses

Ectromelia

Anton M. Allen, Michael J. Collins, Jr., and Frank Fenner

1. **Agent: Ectromelia (Family: Poxviridae; Subfamily: Chordopoxvirinae; Genus: *Orthopoxvirus*). Disease induced: Mousepox.**

   a. **Morphology:** Large, oval to brick-shaped DNA virus (175 x 290 nm) with characteristic dumbbell-shaped nucleoid. Similar to vaccinia and variola.

   b. **Cultivation:** Propagation readily achieved in a variety of cell cultures including L929, Vero, Hela, and BSC. Grows well on the CA membrane of embryonated hen's egg.

   c. **Strains:** Of many known strains (isolates), the Moscow and Hampstead are the most studied. They appear to be indistinguishable antigenically. Enzyme restriction analyses of the genomes have revealed practically no differences.

   d. **Stability:** Relatively stable in ordinary room environment (23 °C), especially under dry conditions. May be preserved for months at 23 °C in glycerol and indefinitely at -70 °C or in freeze-dried state.

   e. **Inactivation:** Relatively resistant to dry heat and to ether. Although rapidly inactivated by moist heat, not all virus particles are consistently inactivated in serum heated 30 minutes at 56 °C. Effective disinfectants include sodium hypochlorite (1,000 ppm of available chlorine), iodophores (150-300 ppm), and vapor phase formaldehyde (paraformaldehyde 10 g/m³).

2. **Characteristics of infection**

   a. **Animals:** Infections are known to occur naturally only in mice, although some reports suggest that certain wild rodents may be inapparently infected. Efforts to infect other laboratory animals experimentally have resulted in brief noncontagious infections with antibody production. Clinical disease is severest in certain strains of mice, especially A, CBA, DBA, C3H, and BALB/c. The C57 Black strains are relatively resistant and often have inapparent infections.

   b. **Geographic distribution:** Infections have been reported in many countries worldwide.

   c. **Means of spread:** Ectromelia is transmitted to mice naturally by exposure to contaminated environment. The primary route of infection is through skin abrasions. Approximately 10 days after infection, characteristic skin lesions of mousepox develop which shed additional virus into the environment. The virus is spread from cage to cage principally by animal handlers, and between laboratories and institutions mainly by researchers who exchange mice and mouse tissues.

   d. **Morbidity and mortality:** Clinically evident disease in affected colonies may vary from 3 to nearly 100 percent depending on factors such as the strain of virus involved, the strain of mouse, the methods of caging and animal care, and the duration of infection in the colony. In slowly progressive outbreaks, the disease may be localized for long periods in a small area of an animal room and require extensive testing for detection. In explosive outbreaks involving highly susceptible mice, illness may be observed in most of the mice and mortality may exceed 80 percent.

   e. **Prevalence:** The prevalence of mousepox in different colonies is influenced by the same factors that affect morbidity and is therefore highly variable and difficult to predict. Since serum antibody is detectable much longer than the disease, prevalence based on positive serology is especially influenced by the duration of the outbreak. After major outbreaks in previously unexposed colonies, prevalence based on the finding of antibody in survivors may approach 100 percent.

f. Pathology: The lesions of acute mousepox include extensive necrosis of the spleen, lymph nodes, Peyer's patches, thymus, and liver. Characteristic large eosinophilic cytoplasmic inclusions occur in epithelial cells of skin at sites of focal epidermal hyperplasia and erosion. These "type A" inclusions may be found also in erosive mucocutaneous and intestinal epithelium. Jejunal hemorrhage commonly results from small punctate mucosal erosions. Basophilic "type B" inclusions are found in the cytoplasm of all infected cells. They are especially prominent in hepatocytes in and adjacent to areas of hepatic necrosis. Mice that survive the more acute stages of the disease may undergo necrotic amputation of one or more limbs. Hence the original name of the disease, "infectious ectromelia."

g. Diagnosis: Acute mousepox usually can be diagnosed on the basis of finding typical lesions. Confirmation is easily accomplished by demonstration of the large virus in affected tissues electron microscopically. Other methods include virus isolation in mice, eggs, or tissue culture and demonstration of viral antigen in tissues by immunocytochemical techniques. Serologic confirmation is useful in mice that have survived acute infection.

h. Interference with research: Ectromelia ranks with Sendai and mouse hepatitis viruses as a major cause of interference with research. Colonies of susceptible mice may be decimated on first contact with highly virulent strains of virus. In partially immune populations or resistant strains of mice, inapparent infections may cause unexplainable data variation and misinterpretation of experimental results. Also, measures used to control and eradicate mousepox usually cause severe disruption of research.

3. Control/prevention

a. Special considerations: Eradication of mousepox usually requires elimination of the affected colonies, disinfection of rooms and equipment, and disposal of all infected tissues and sera. Frozen tissues may be tested for ectromelia by virus isolation or the mouse antibody production technique.

b. Effectiveness of vaccines: Vaccination with vaccinia virus may be used to eliminate infection in small closed colonies. This method is not recommended for large production colonies since strict quarantine would be difficult to maintain, and the whereabouts of many offspring would have to be traced continuously in order to vaccinate them at 5 to 6 weeks of age. For vaccination, a live egg-adapted strain of vaccinia virus is used. It gives strong cross-protection and, if the IHD-T strain is used, no hemagglutination-inhibiting antibody is produced, thus allowing for use of the HI test for serologic screening. Vaccination, however, does interfere with the use of the more sensitive ELISA and IFA tests.

c. Effectiveness of hysterectomy derivation: Rederiving of infected mouse stocks by this means cannot be relied upon to eliminate ectromelia virus since intrauterine infection is common in mice infected when pregnant. Although the fetuses die, the virus may be introduced into the clean area with the uterine contents.

4. Test Procedures

a. Isolation: Ectromelia virus may be isolated from extracts of liver and spleen of diseased mice by inoculating susceptible mice, the CA membrane of fertile hens eggs, and many types of cell cultures including KB, Vero, Hela, L929, B56, and primary cultures of chick embryo. Identification is made by HA-HI, IFA, presence of inclusion bodies, or electron microscopy.

b. Serology: Because of safety considerations, ectromelia virus should not be used. All antigens employed in serological tests should be derived from recognized strains of vaccinia virus.

ELISA is suitable for screening large numbers of sera quickly. It is 10- to 100-fold more sensitive than the HI, but not as specific as the IFA. False positives are

sometimes a problem with certain lots of test plates and when sera contain anti-DNA antibodies as in NZB mice. Antigen for the ELISA is prepared from cell cultures of nonmouse origin.

HI is suitable for screening large numbers of sera but is relatively insensitive especially in enzootic infections. Unexplained false positive HI titers are occasionally found in certain groups of mice. It is the only serological test for ectromelia that does not give a positive reaction with sera from mice vaccinated with the IHD-T strain. HA antigen is extracted from either vaccinia virus infected CA membranes of 10-day-old fertile hens eggs or from cell cultures. Four units of antigen are used in the HI test. Chicken rbc's must be pretested to assure sensitivity to vaccinia virus HA antigen. Mouse sera often contain agglutinins and should be absorbed. Sliding the settled rbc's helps determine weakly agglutinated patterns. Titers of 1:20 or greater are considered positive; however, a 1:10 titer can be significant in an epizootic.

IFA is the most sensitive of the serological tests employed and permits visualization of the antigenic sites. It is used mainly as a confirmatory procedure rather than for screening because it is time-consuming and cumbersome to conduct. Antigen for the IFA test is vaccinia virus infected cell cultures of nonmouse origin grown on cover slips and fixed in acetone.

Vaccinia virus antiserum is produced in virus free mice given intraperitoneal injections of extracts of vaccinia virus infected L-929 cell cultures. Enzyme conjugated and fluorescin conjugated rabbit antimouse IgG are purchased from commercial sources. All reagents employed in serological tests should be screened against a known battery of sera and antigens. Detailed descriptions of reagent preparations and performance of the tests have been published (Briody; Christensen et al.; Collins et al.; Voller et al.).

c. Histopathology: (See **"Pathology"** above.) In susceptible strains of mice, histopathology may be used as a supplemental screening method. The lesions of acute mousepox are characteristic and provide basis for presumptive diagnosis by an experienced pathologist.

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Hantaan and Related Viruses

James W. LeDuc, Karl M. Johnson, and Junichi Kawamata

1. **Agent: Hantaan and related viruses (Family Bunyaviridae, proposed new genus, *Hantavirus*). Disease induced: Hemorrhagic fever with renal syndrome.**

   a. Morphology: RNA viruses with spherical to oval particles having a unit membrane and subunit structure, average diameter 95 nm (range 80-115 nm).

   b. Cultivation: Propagation in Vero E-6 cells and A-549 (continuous human lung carcinoma cell line) is detected by immunofluorescence. Viral antigen is restricted to the cytoplasm and appears in a discrete granular pattern; noncytolytic, persistent infection is produced. Primary isolation of virus strains in cell culture is difficult.

   c. Strains: Four distinct viruses comprise the proposed genus **Hantavirus:** Prototype Hantaan virus, strain 76-118 isolated from *Apodemus agrarius coreae* (striped field mouse) in Korea; Seoul virus (also known as urban rat-associated Hantaan-like virus), strains Girard Point, Tchoupitoulas, SR-11 and others, all isolated from **Rattus;** Puumala virus isolated from **Clethrionomys glareolus** in Scandinavia; and Prospect Hill virus isolated from **Microtus pennsylvanicus** in USA. Hantaan virus is the etiologic agent of Korean hemorrhagic fever in Korea, epidemic hemorrhagic fever in Japan and China, and perhaps "severe" hemorrhagic fever with renal syndrome (HFRS) of the USSR. Seoul virus causes "mild" HFRS in Korea and China and is probably responsible for most laboratory rat-associated outbreaks of HFRS. Puumala virus causes neophropathia epidemica of Scandinavia, western USSR and western Europe. Prospect Hill virus is not known to cause human disease.

   d. Stability: Hantaviruses are relatively stable at low temperatures and neutral or slightly alkaline pH. They may be stored at -70°C; no significant drop in titer occurs with overnight incubation at 4°C.

   e. Inactivation: Hantaviruses are rapidly inactivated under acid conditions ($\lesssim$6.0) and high temperature; effective disinfectants include sodium hypochlorite and phenolics. They are inactivated by gamma irradiation.

2. **Characteristics of infection**

   a. Animals Clinically inapparent persistent hantaviral infections are known to occur in a variety of strains of rats. Natural infections in wild rodents (*Apodemus* in Korea, *Clethrionomys* in Scandinavia and USSR, *Peromyscus* and *Microtus* in US) with hantaviruses have been reported. Urban rats (*R. rattus* and *R. norvegicus*) captured in major Asian and Western cities have been found infected with hantaviruses (Seoul Virus). Susceptibility of laboratory mice has yet to be clearly established.

   b. Geographic distribution: Outbreaks among persons in direct contact with infected laboratory rat colonies have occurred in Korea, Japan, Belgium, France, and the UK. Sera from urban rats captured in major cities in Korea, Japan, Thailand, Burma, Egypt, Brazil and the US react serologically with hantaviruses. Epidemic hemorrhagic fever of Japan, various forms of hemorrhagic fever with renal syndrome in China and USSR, and nephropathia epidemica of Scandinavia are thought to be caused by hantaviruses (see **"Strains"** above).

   c. Means of spread: Mode of transmission is incompletely understood; however, spread between laboratory rats and to humans in the laboratory setting is thought to be via aerosol or bite. Infectious virus has been found in urine, feces, and saliva of infected rats. Rats become persistently infected and shed infectious virus for extended periods, perhaps for the duration of their lives. Persistence of infectious virus in animal bedding has not been established, but should be considered potentially hazardous pending additional

studies. Transmission between rodents and to man in nature has not been defined, but aerosol transmission is suspected. Transmission by arthropod vectors has been suggested but not definitely established and is probably of limited importance.

d. Morbidity and mortality: Clinical disease among laboratory rodents infected with hantaviruses has not been documented. Significant risk of overt disease exists among humans exposed to infected animal colonies, but attack rates among such persons are unknown.

e. Prevalence: Antibody prevalence rates in infected colonies have not been studied in depth, but isolated instances of >80 percent antibody positive individuals have been found.

f. Pathology: Undetermined.

g. Diagnosis: Hantaan virus infection is diagnosed by demonstration of specific anti-Hantaan virus antibody in infected hosts. All recognized hantaviruses elicit antibody which is reactive with prototype Hantaan virus antigen. Large scale screening is best conducted using immunofluorescence, radioimmunoassay, or ELISA techniques. Confirmation of screening results is best done by plaque reduction neutralization tests on either Vero E-6 or A-549 cell cultures.

Both immunoglobulin G and M are elicited following infection with hantaviruses.

Characteristic viral antigen can be detected in organ tissues by immunofluorescence in approximately 30 to 50 percent of the antibody positive individual rodents.

Antigen is generally most abundant in lung tissues, but also may be detected in spleen, kidney, and other tissues.

h. Interference with research: Hantaviral infection in laboratory rat colonies represents a significant health hazard to persons exposed to the colonies, including both caretakers and professionals. Disease among persons infected from contaminated rat colonies ranges from mild flu-like symptoms to life-threatening hemorrhagic disease and occasionally death. One clearly documented fatal case has been recorded among 141 persons infected while working with infected rat colonies in Japan. The mortality rate among humans infected in the endemic areas of Korea is about 5 percent, while among nephropathia epidemica patients in Scandinavia it is less than 1 percent.

Since hantaviruses establish persistent, inapparent infections in rats, the potential exists for such infection to produce confounding experimental results. The degree to which this might have occurred is currently unknown.

3. Control/Prevention

a. Special considerations: Standardized procedures for the eradication of hantaviruses from affected colonies have not been established; however, elimination of affected colonies, disinfection of rooms and equipment, and disposal of all infected tissues and sera seem appropriate, considering the difficulties in diagnosis and containment of the infection.

b. Effectiveness of vaccines: No vaccine is available for hantaviruses.

c. Effectiveness of hysterectomy derivation: Well controlled studies to evaluate the efficacy of eliminating hantaviruses from infected colonies have not been conducted. Preliminary results from two separate laboratories suggest that this technique may have merit. Pregnant *Apodemus* experimentally infected with Hantaan virus delivered normal offspring.

4. Test Procedures

a. Isolation: Seronegative rodents may be inoculated with suspect contaminated tissues and subsequently examined for the development of anti-hantavirus antibody. This procedure yields a high rate of "isolations" when inoculated tissues contain immunofluorescent hantaviral antigen. Subsequent rodent-to-rodent and rodent-to-cell-culture passage is possible.

Hantaviruses are extremely difficult to isolate directly and no method of isolation has been described which consistently yields an isolate. The most successful approach to isolation has involved inoculation of cell cultures (Vero E-6 or A-549) and subsequent examination of cells for characteristic immunofluorescence (CPE is not produced). Incubation for approximately 10-14 days is required prior to examination for antigen, and several blind passages may be necessary prior to antigen detection. The substrate which has most frequently yielded virus isolations is lung suspensions from infected rodents. Lee reported approximately 10 percent success rates in attempts to recover Hantaan virus from antigen positive rodent tissues inoculated into cell cultures (pers. comm. H.W. Lee, WHO Collaborating Centre for Research on Korean Hemorrhagic Fever, Korea University).

b. Serology: Serological tests available are detailed under "**Diagnosis**" (2.g.) above.

c. Histopathology: Characteristic histopathologic lesions of hantaviral infection in rodents have not been described.

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Lymphocytic Choriomeningitis Virus

John C. Parker

1. Agent: Lymphocytic choriomeningitis (LCM) Virus (Family: Arenaviridae; Genus: *Arenavirus*). Disease induced: Lymphocytic choriomeningitis.

a. Morphology: Round to pleomorphic varying in diameter from 50 to 300 nm. The surface of the virion is covered with club-shaped projections 10 nm long. Virions are formed by a budding process with the outer cellular membrane transformed into the viral envelope. The interior of the particles contain one to several granule-appearing ribosomes from which the family name is derived. The RNA is single stranded and consists of five segments (31S, 28S, 22S, 18S, and 4-6S). Of these, only the 31S and 22S are virus specific. The 28S, 18S, and 4-6S segments are all derived from the host ribosomes incorporated into the virion upon budding from the infected cell.

b. Cultivation: The virus replicates in many cell cultures including mouse, hamster, monkey, bovine, and human. Vero, L-929, and BHK-21 cells are frequently used for preparation of antigen or isolation and titration of virus. Infection in cell culture may be brief, with or without CPE, or may be prolonged as a persistent infection, the outcome thought to be influenced by the virus strain, type of cells, and initial multiplicity of infection. In vivo cultivation can be made in mice, hamsters, or guinea pigs with strain of virus, animal species, route of inoculation, and age of the animal having profound effects on the outcome of infection. Intracranial inoculation of weanling mice is the preferred in vivo method for preparation of virus antigen and isolation of virus. In vitro cultivation in MDCK cells leads to lytic infection, whereas infection of most other cell lines results in persistent infections. The most frequently used cell lines are Vero, L-929, and BHK.

c. Strains: The most commonly used laboratory strains are Armstrong (CA1371), E-350, WE, Traub, and Fortner. Strains may vary widely in their pathogenicity for different species, especially man. The passage history of isolates has a significant effect on altering the biological properties of the virus strain. A strain of virus may show different characteristics when inoculated by different routes or when infecting different species or different ages.

d. Stability: Relatively unstable; very thermolabile but can be partially stabilized by protein additives. May be preserved indefinitely at -70°C or by lyophilization.

e. Inactivation: Inactivated at pH below 5.5 and above 8.5, at 56°C, and by lipid solvents. Highly sensitive to UV and gamma irradiation, soaps, and detergents.

2. Characteristics of infection

a. Animals: Natural LCM infections occur most commonly in wild and colony-reared mice and less frequently in hamsters and man. Guinea pigs, monkeys, dogs, field mice, and newborn rats can be infected experimentally.

Transmission in mice is primarily vertical by the in utero route, which results in a persistent inapparent infection. The virus carrier mouse provides the principal mechanism for perpetuation of the infection in the natural state. This persistent infection is characterized by the consistent presence of high titers of virus in the blood and other tissues of the mouse and no easily discernible tissue damage until late in life. Antibody in virus carrier mice can be detected by solid-phase RIA.

The acute disease leading to death can be produced by inoculation of immunocompetent mice by the intracranial route. Weanling mice contracting the infection by a natural route have uneventful infections and clear virus within a few weeks.

Thus, the course of infection and type of disease is influenced by the age and strain of the mouse and route of infection. Four types of disease may be produced: cerebral disease, visceral disease, runting disease, or late onset wasting disease.

Hamsters infected congenitally or neonatally may appear healthy while excreting virus at high titer for a few to many months before the infection either clears or the animals develop disease and die. The course and outcome of infection is dependent on the age and route of infection, and the strain of virus (see **"Morbidity"**).

b. Geographic distribution: Worldwide in colony-reared and wild mice but not reported in all countries, notably Australia and Africa.

c. Means of spread: The primary method of virus dissemination in mice is in utero vertical transmission resulting in persistent lifelong infection (see **"Animals"**). Dams transmit the virus to their offspring with an efficiency of 100 percent. While virus is excreted into the urine and saliva, contamination or biting is not a principal method of horizontal transmission in the mouse.

Hamsters and mice are the only animals that readily transmit the infection to other species. Human infections are self-limiting. Vertical infection does occur in hamsters but with less efficiency than in mice. In contrast to the mouse infection, hamster infection is characterized by high titer viremia and viruria, and virus spread to other hamsters occurs with great frequency. Infected hamsters provide a high degree of risk of infection to humans through contact with the animals or contaminated bedding. Because of the great lability of the virus, spread by dust aerosols and survival on dry surfaces is not likely.

d. Morbidity and mortality: Clinical evidence of infection in infected mouse colonies is normally absent. A few infected older mice may develop wasting disease, but this is not pathognomonic for LCM and is easily overlooked. Neonatally or vertically infected hamsters that do not clear their infections show clinical evidence of infection as early as 2 to 3 months of age and frequently by 6 months. In the earliest stages, the hamsters become less active and they appear ungroomed. Later the animals begin to lose weight, assume a hunched posture, and develop blepharitis leading ultimately to a wasting type syndrome and eventually death. Mature mice or hamsters acquiring the infection horizontally do not show clinical evidence of infection.

e. Prevalence: Laboratory and commercially reared colonies of mice are rarely found to be infected with LCM virus; less than a dozen have been reported in the US in the past decade. When an outbreak does occur, several colonies of animals and sometimes humans become involved.

Within infected wild mouse colonies, prevalence may be quite high because of inbreeding and vertical transmission, but in colony reared mice prevalence is low. Usually less than 10 percent are infected and less than 5 percent have detectable antibody, indicating a low degree of spread within infected colonies (or an insensitive serological test). Prevalence rates in hamsters may be higher because of increased environmental contamination, hence greater likelihood of contact with virus. The infection in both mice and hamsters may not be diagnosed because of the absence of clinical signs, difficulty in detecting circulating antibody by serology, and low prevalence rates in mice.

f. Pathology: Immunopathological disease and death occur in adult mice following intracerebral inoculation; however, when mice are infected before or shortly after birth they develop a relatively nonpathogenic lifelong persistent infection. The disease in weanling mice following intracerebral inoculation is characteristic: evidence of infection is not seen for the first 5 days; from the 6th to 9th day clinical signs appear which include ruffled hair, hunched backs, and partially closed eyes. Convulsive attacks with tremors of the head and extremities and tonic extension of the hind legs leading to death characterize the acute disease. Extraneural infection of adult mice less often leads to neurologic

disease. Infection may be inapparent by the footpad route or severe by the intraperitoneal route with ruffled hair, conjunctivitis, lethargy, and death and is influenced by strain of virus, age of the mouse, and dose of virus. Persistent infections are asymptomatic until near or after 1 year of age, when a chronic illness may develop, characterized by ruffled hair, hunched posture, and weight loss. Both chronic and acute infection with consequent disease are immunopathologic. The predominant manifestation is lymphocytic infiltration around the blood vessels of the meninges and choroid plexus. Glomerulonephritis, especially in hamsters, is characteristic in the late stages of persistent infections. Protective immunity, footpad response, disease, and virus elimination have all been shown to be cell-mediated immune phenomena.

g. Diagnosis: In the mouse, histopathology and clinical disease are of little value in diagnosis because of the relative absence of these features in the persistently infected animal. Serology is of limited value and must be used selectively with foreknowledge of the biology of the infection and the species involved. In persistently infected mice, immunosuppression of both humoral and cellular responses occurs and therefore serologic tests (complement fixation (CF), neutralization, immunofluorescence (IFA)) are of little value in diagnosis. However, by use of the solid phase RIA test, antibody in virus carrier mice can be detected easily. In a mouse colony where immunocompetent animals become infected these tests are quite sensitive and reliable; however, since spread of infection is limited, large numbers of animals must be tested. IFA is useful for detecting immune complexes in the kidneys of infected mice and hamsters. Serodiagnosis using CF, IFA, and neutralization tests may be used effectively for the hamster, guinea pig, human, and monkey. The IFA test is the most sensitive and reliable assay for human infection. The virus strain and antigen preparation is important in serology.

The recommended method for diagnosis of infection in mouse colonies is virus isolation or detection of antibody in immune complexes. Inoculation of blood, body fluids, or tissue homogenates into LCM virus-free mice by the intracranial route may induce neurologic disease and death within 6 to 9 days. Surviving mice may be challenged with a known lethal dose of LCM virus which will kill the nonimmune mice, whereas the mice that were immunized by a sublethal dose in the test sample will survive. Direct challenge of test mice with lethal doses of LCM, use of cell culture isolation coupled with IFA or plaque formation, and footpad inoculation with subsequent challenge by lethal doses are other assays that have value.

h. Interference with research: The major consequences of inadvertent LCM infection in rodents is the serious health hazard posed to humans. In addition, immunologic research may be compromised if persistently infected (PTI) mice are used. While apparently normal with respect to B cell function, these PTI mice have no cytotoxic T cells and thus an apparent defect in effector T^1 cell function. Also, because of the viremia and wide distribution of virus in all tissues of PTI mice, any use of these infected mice for production of reagents (antigen, antibody, cell culture, etc.) or tumor passage would almost certainly result in their contamination with LCM virus.

3. Control/prevention

a. Special considerations: Elimination of LCM infection requires the destruction of the entire colony and decontamination of the physical structure and equipment. Reagents, tissues, or tumors derived from or passaged through these animals may be contaminated and should be tested for virus by the mouse antibody production procedure.

b. Effectiveness of vaccines: Vaccines would be of no value in eliminating infection.

c. Effectiveness of hysterectomy derivation: LCM virus infection in mice and hamsters may be transmitted vertically in the female by the transovarian or transuterine route; this precludes hysterectomy as an effective method for rederivation. To circumvent this problem, females may be pretested for

viremia and if negative can be used for rederivation under proper conditions of isolation and protection from infection prior to rederivation. Infected spermatozoa have not been implicated in transmission.

4. Test Procedures

a. Isolation: Virus may be isolated from blood or tissues at any time during the life of persistently infected mice (see **"Diagnosis"**). Virus may be demonstrated in infected cell cultures by IFA (see **"Cultivation"**) or in mice inoculated by the intracranial route. Indirectly, virus may be demonstrated by the mouse antibody production test, which consists of inoculation of mice and later demonstration of antibody in the mice induced by the presence and replication of the virus.

b. Serology: The three most frequently used serological tests are complement fixation (CF), indirect immunofluorescence (IFA), and neutralization. Serology is of no practical value in attempting to detect virus carrier mice as they do not have easily demonstrable CF, IFA, or neutralizing antibody. Immunocompetent species develop antibodies that can be detected by all three assays. The most frequently used assay to detect infection in hamsters is CF, while IFA is the test of choice for humans. The IFA is probably the most sensitive and most useful test for the serological diagnosis of LCM infection in all species when infection occurs in the immunocompetent animal.

The LBCF (Laboratory Branch Complement Fixation Test, CDC) procedure employing five CH50's complement is the most sensitive CF procedure. CF antigen must be block titrated to select the optimal use dilution, as zoning problems are particularly troublesome in LCM CF tests. The strain of virus is also important, and it is often advisable to use different strains and cell culture systems (Fortner and CA1371) when confirmation of equivocal results is required. Antibody titers are normally low ranging between 1:10 and 1:40. A low-level cross-reaction is observed with Lassa fever virus and some of the Tacaribe complex antisera against LCM antigen. Antigens involved with neutralization are strain specific within the *Arenavirus* genus. By IFA, all Tacaribe complex viruses and Lassa fever virus cross-react with LCM.

IFA tests use infected cell culture monolayers, prepared on coverslips and fixed and stored at -70°C until used. Test serum is overlaid onto the cells and washed off, and the antispecies conjugated antiserum (fluorescein isothiocyanate-conjugated anti IgG or IgM) overlaid onto the cells. Positive immunofluorescence consists of a granular cytoplasmic fluorescence.

Neutralization tests may be performed in either cell culture or in mice. The test is less sensitive than CF or IFA in human diagnosis and infrequently used for diagnosis in rodents.

c. Histopathology (see also "Pathology"): Acute LCM disease following inoculation of virus consists essentially of meningoencephalitis and hepatitis and lymphocytic infiltration of all organ systems of the animal, especially the liver, adrenals, kidneys, lungs, and meninges. Glomerulonephritis and vasculitis are consistent sequelae in persistent infections in the hamster and in wasting disease of older LCM-infected mice.

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Mouse Hepatitis Virus

Kosaku Fujiwara and Joseph E. Wagner

1. **Agent: Mouse hepatitis virus (MHV) (Family: Coronaviridae; Genus: *Coronavirus*). Disease induced: mouse hepatitis.**

   a. Morphology: Pleomorphic spheroidal enveloped RNA virus 80 to 160 nm in diameter with characteristic clubshaped spikes or peplomers about 20 nm long by 7 nm wide at the tip.

   b. Cultivation: Growth with or without syncytium formation is obtained in primary cultures of mouse macrophages and some cell lines derived from mouse tissues such as the liver (NCTC 1469) or fibroblast line (L929). Some transformed cell lines are also useful such as DBT, AN, BALB/c 3T3 and 17CL1. In the absence of CPE, viral antigen can be easily detected by IFA.

   c. Strains: Many strains that differ in pathogenicity and antigenicity have been isolated. Some such as MHV-2, MHV-3, and MHV-A59 are more virulent than MHV-1, MHV-S, MHV-Y and MHV-Nu, which are less virulent. MHV-4 (JHM) is neurovirulent. The pathogenicity also depends on the genotype and age of the mouse. Recent findings suggest that MHV strains fall into two groups according to their predominant tissue tropism—those that replicate mainly in the respiratory tract (MHV-1, -3, -5, -JHM, -A59; Tettnang; wt -1) and those that replicate mainly in the intestinal tract (MHV - Y, wt -2). Involvement of other organs such as the liver, brain, bone marrow and lymphoid organs is considered to be secondary.

   d. Stability: Survives well at 60°C in protein-fortified medium. Is more stable at pH 6.0 to 6.5 than at pH 8.0 when kept at 37°C. It is moderately resistant at pH 3.0 and to sodium desoxycholate. Survives well at −70°C. Is comparatively unstable after lyophilization.

   e. Inactivation: The virus is inactivated at 56°C in 30 minutes. It is sensitive to ether and chloroform.

2. **Characteristics of infection**

   a. Animals: Natural infections occur only in mice. The clinical picture depends on mouse genotype, age, route of infection, and the virus strain. BALB/c and C57BL mice are highly susceptible to mortality while C3H are relatively resistant. Most immunocompetent adult mice show very little clinical disease with natural infections. Infected nude mice develop a chronic wasting disease with or without hepatitis.

   b. Humans: None. Antibodies in man are due to infection with human coronavirus strains.

   c. Geographic distribution: Worldwide.

   d. Means of spread: Primary route of natural transmission appears to be oral or oral-nasal. Virus replicates in nasal epithelium. Contact with an infected mouse as well as contaminated fomites (bedding, feces) are sources of transmission in a colony.

   e. Morbidity and mortality: With wild strains of MHV generally there is low morbidity and little mortality in adult outbred mice. The response of the inbred strain is genotype-dependent. Morbidity and mortality are high in infant mice in the absence of maternal antibody. Signs due to central nervous system (CNS) involvement (encephalitis, demyelination) may be observed in colonies of infected mice with a neurotropic virus strain. Similarly, nude mice suffer from high morbidity and mortality.

   f. Prevalence: Infection results in seroconversion in all strains of mice. Information on virus persistence in a colony in the absence of introduction of susceptibles (adults or infants in the case of a breeding colony) is not known. Information about reinfection with the

homologous or heterologous strain of virus is not available. Antibody persists for a lifetime.

g. Pathology: Lesions may occur in different organs depending on the virulence and tropism of the virus strain. Grossly, the liver may be pale and have multiple small white spots, petechea, or depressions, and the bowels may be dilated and filled with gas and fluid. The spleen often is enlarged and the thymus reduced in size. Microscopically, focal necrosis, subacute to chronic inflammatory cell infiltration, and even syncytial formation may be seen in the liver. Lymphoid tissue also may undergo necrosis. Suckling mice with diarrhea are shown to have severe enteritis with syncytium formation in enterocytes. Neurotropic strains produce encephalitis.

h. Diagnosis: Acute fatal infection can be diagnosed on the basis of typical lesions (liver, intestines, nasopharynx) and positive indirect immunofluorescent test for viral antigen. Virus can be isolated in infant mice or tissue culture, but the results do not always correlate with IFA results. The MAP test can also detect virus antigen. Antibody in the mouse colony can be detected by ELISA or IFA test. The CF test is insensitive.

i. Interference with research: Morbidity and mortality in infant mice, nude mice, and adult mice of certain genotypes cause overt interference. Infections modulate immune response with resultant variation in research data. Liver metabolism also is affected. This infection can contaminate products obtained from an infected mouse such as tumor transplants, ascitic fluid, and virus pools prepared from brain, liver, spleen, and bone marrow cells.

3. Control/prevention

a. Special consideration: There is wide variation in clinical disease due to this virus; therefore sensitive antibody tests are the only reliable indicators of infection. There is paucity of information about the duration of infectiousness of an infected mouse. This will be affected by the genotype and age of the mice. In recent years it has been difficult to control and prevent this infection. Use of filter caps on cages and/or the housing of mice in laminar air flow caging systems or hoods has been effective in preventing introduction of the virus into colonies and subsequent spread. Rigid protocols must be used to prevent introduction of the virus into these protective environments at the time of experimental or other manipulation of the mice.

b. Effectiveness of vaccines: Vaccines against mouse hepatitis virus are not currently available. However, it has been shown experimentally that vaccination with formalin-inactivated virus from affected mouse liver or DBT cell culture is effective enough to protect mice from challenge infection with virulent MHV. Immunization of dams with the same vaccine produces neutralizing antibodies transferable in milk to offspring, conferring in them resistance to enteritis due to MHV. Nude mice cannot be immunized by vaccination because the immune response to MHV requires T cells.

c. Effectiveness of hysterectomy derivation: There is no evidence of intrauterine transmission of MHV to the fetus under natural conditions, but this has been reported under experimental conditions. Therefore appropriate monitoring of hysterectomy-derived mice is necessary before releasing them to clean mouse colonies.

4. Test procedures

a. Isolation: MHV may be isolated in susceptible tissue culture cells—either primary or continuous cell lines (see **"Cultivation"**). NCTC1469 and DBT are commonly used. Mouse inoculation, particularly infant mice from a colony free of MHV infections, may be as sensitive as tissue culture or even more sensitive for MHV isolation. In older mice, cortisone treatment may be required to enhance infections. A judicious use of the MAP test with an amplification step (passage in suckling mice) may be useful to detect virus.

b. Serology: ELISA is sensitive and relatively specific for detection of antibody. Indirect immunofluorescence test is also a sensitive and relatively specific test. It is important to use as antigen a broadly cross-reactive virus that will detect antibody to various strains of MHV. CF test is unsatisfactory as it is very sensitive to variation in complement concentrations. Antibody response to the same strain of virus is known to be different among mouse strains.

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Rat Coronavirus/Sialodocryoadenitis Virus

Robert O. Jacoby and Pravin N. Bhatt

1. **Agent: Rat coronavirus (RCV)/Sialodacryoadenitis virus (SDAV), currently viewed as strains of the same rat coronavirus (Family: Coronaviridae; Genus: *Coronavirus*). Disease induced: Sialodacryoadenitis and coronavirus pneumonia.**

   a. **Morphology:** Large, pleomorphic, single-stranded RNA virus with a characteristc corona of bulbous peplomers. Particles measure 60 nm to 200 nm in diameter.

   b. **Cultivation:** Causes cytopathic effect characterized by syncytia formation in monolayer cultures of primary rat kidney cells. Cultures should be inoculated by 1 week after seeding because older cells are less susceptible to viral replication. Viral antigen can be detected by 12 hours, syncytia formation by 24 hours, and lysis by 36 to 48 hours. A Japanese isolate has been propagated in 3T3 cells.

   c. **Strain:** All field and laboratory strains tested are antigenically similar. Share complement fixing antigens with mouse hepatitis viruses.

   d. **Stability:** Sensitive to lipid solvents, but relatively stable to acid pH (3.0). Also stable in phosphate-buffered saline with 3 percent fetal bovine serum at 4°C for up to 7 days and at 37°C for up to 3 hours. Virus is inactivated at 56°C in less than 5 minutes. Can be stored indefinitely at −60°C, but loses infectivity rapidly at −20°C.

   e. **Inactivation:** Chemical disinfectants such as sodium hypochlorite (525 ppm) or lipid solvents will inactivate rat coronaviruses rapidly.

2. **Characteristics of Infection**

   a. **Animals:** Natural infection appears to be restricted to rats. Rats of all ages are susceptible. Variations in susceptibility among inbred strains has not been examined systematically. Experimental inoculation of mice produces mild, transient interstitial pneumonia and seroconversion. Mouse to mouse contact-exposure also can evoke seroconversion.

   b. **Geographic distribution:** Worldwide.

   c. **Means of spread:** The primary route of transmission appears to be respiratory. Virus can spread quickly by aerosol or contact exposure. Virus is excreted from salivary glands for about 7 days after primary infection. It has not been detected in urine or feces. Lacrimal excretion is likely. Intrauterine transmission has not been demonstrated.

   d. **Morbidity and mortality:** Morbidity is high and mortality is insignificant. Epizootics often infect 100 percent of exposed animals, although only a portion may have clinical signs. Enzootic infection is characterized by disease among naive animals. In enzootically-infected breeding colonies clinical signs are most common in weanlings when maternally-acquired immunity has decayed. Infection is self-limiting in individual rats and produces long-lived immunity to reinfection. Reinfection, especially in aged rats, cannot be ruled out because the maximum duration of acquired immunity has not been established.

   e. **Prevalence:** Infection evokes prominent, longstanding seroconversion in essentially all rats. Virus does not persist in a colony once all animals are immune. Primary infections and reinfections are associated with spiking of complement-fixing antibody titers whereas neutralizing antibody titers tend to plateau, then slowly decline.

   f. **Pathology:** Acute lesions occur in serous and mixed salivary glands, lacrimal glands and the respiratory tract. Infection begins with mild necrotizing rhinotracheitis which may be followed by transient interstitial pneumonia. Peribronchial lymphoid hyperplasia is common. Salivary and lacrimal gland lesions are characterized by severe necrosis and

inflammation which begins in the excretory ducts and quickly spreads to acinar parenchyma. Restoration of cytoarchitecture begins in about one week and is characterized by squamous metaplasia which is especially prominent in the Harderian lacrimal glands. Glandular involvement may be unilateral or bilateral and proceeds to full recovery in 3 to 5 weeks. Remnants of glandular disease are limited to occasional interstitial lymphocytic infiltrates and mild fibrosis. Long term sequellae of infection involve the eye and are commonly expressed as keratoconjunctivitis. This lesion usually occurs in fewer than 10 percent of infected rats and varies from a mild, self-limiting condition to severe progressive anterior ophthalmitis with corneal ulceration, megaloglobus, and pressure atrophy of the retina.

g. Diagnosis: Coronavirus infection is often accompanied by one or more of the following clinical signs: high morbidity, photophobia, lacrimation, excessive porphyrin secretion ("red tears," "bloody nose"), cervical swelling, inappetance, weight loss, sneezing, corneal opacities, megaloglobus, hypopyon, hyphema. Clinical signs are augmented by characteristic lesions and by seroconversion. Viral antigen can be demonstrated in affected tissues by immunocytochemical methods during the first week of infection. Virus isolation is feasible, but not usually necessary.

h. Interference with research: During acute disease rats may be anesthetic risks because of inflammation-mediated obstruction of airways. Inappetance and cervical pain may complicate feeding studies. Respiratory lesions may increase susceptibility to opportunistic infection with other microorganisms and may interfere with inhalation toxicology research. Ocular lesions can compromise ophthalmological studies.

3. Control/Prevention

a. Special consideration: Because rat coronavirus infection spreads rapidly and is self-limiting, strict quarantine is a simple, effective method to promote immunity and eliminate virus. Cessation of breeding for 3 to 5 weeks is recommended to eliminate continuous introduction of susceptible animals (sucklings, weanlings) that can perpetuate infection. The immune status of a colony can be followed serologically. Naive animals should not become infected if they are exposed to a solidly immune population.

b. Effectiveness of vaccines: Vaccines against rat coronaviruses are not currently available.

c. Effectiveness of hysterectomy derivation: Hysterectomy derivation can be used successfully to eliminate infection, but given the effectiveness of quarantine procedures for control, hysterectomy has no inherent advantage and is relatively costly and time-consuming.

4. Test Procedures

a. Isolation: SDAV can be isolated from the nasopharynx, trachea, lung, cervical lymph nodes, submaxillary and parotid salivary glands, and exorbital and intraorbital lacrimal glands for up to one week postinfection. RCV is found more commonly in the respiratory tract than in the salivary or lacrimal glands. Nasopharyngeal washes collected 2 to 5 days post exposure are especially useful for isolation of either virus. Tissues should be inoculated into primary rat kidney cell monolayers immediately or be stored at $-60°C$ until cultures are prepared. Cytopathic effects and antigen detection are described above. Alternatively, tissues can be inoculated intransally into naive weanling rats that are examined after 5 to 7 days for lesions and viral antigen or after 3 weeks for seroconversion.

b. Serology: Serum antibody to rat coronaviruses can be detected by one week postinfection. Several serological tests are available. The complement-fixation (CF) test utilizes acetone extracted brain from suckling mice inoculated intracerebrally with SDA virus. Because it is a relatively insensitive test, it is a good marker for recent or active

infection that provokes sharp elevations of serum CF antibody titers. The neutralization test utilizes mouse brain-derived or tissue culture-adopted virus and is more expensive, cumbersome, and time-consuming.

Neutralizing antibody tends to precede CF antibody in serum and also decays more slowly. Therefore neutralization tests are more suitable for retrospective evaluation of a colony. There is some advantage to performing these tests concommitantly to obtain an accurate seroepidemiological history. Two newer tests have recently been developed. An ELISA which utilizes a polyvalent antigen of 4 prototype strains of mouse hepatitis virus appears to be highly sensitive. An immunofluorescence assay using MHV infected L929 cells as a substrate for test sera subsequently stained with fluoresceinated anti-rat antibody also is highly sensitive. These latter tests will require additional field testing before they are accepted for general use. Precautions for performing CF and neutralization tests are as follows:

The CF test should include known positive and known negative controls. The test is very sensitive to complement concentrations. If two or more units of complement are used, false negative reactions may occur. Therefore, 1.5 to 1.8 units of complement are routinely used. Instead of the standard 4 units of antigen, 16 to 32 units of antigen provide better results. For the neutralization test, serum diluted 1:10 is added to an equal volume of virus containing approximately 100 $TCID_{50}$/0.1 ml. After 1 hour incubation at 37°C, four primary rat kidney culture replicates are inoculated with the virus serum mixture. The test is read on days 3 and 5.

c. **Histopathology:** Salivary and lacrimal lesions of rat coronavirus infection are characteristic. Because lesions may be unilateral, sampling both members of paired organs is indicated. There is some experimental evidence that rat coronavirus is not as adenotropic as SDA virus, but this impression has not been thoroughly confirmed. Similarly, adenotropism in individual rats may vary, so all commonly infected tissues should be examined. Typical lesions run a course of about 3 weeks and the reparative phase, characterized by squamous metaplasia, is usually detected by the end of the first week postinfection.

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Rat Parvovirus

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1. Agent: Rat Parvovirus (Family: Parvoviridae; Genus: *Parvovirus*).

a. Morphology: Small (18-30 nm), icosahedral, nonenveloped DNA virus with a linear single stranded genome of 1.35 to 1.70 megadaltons.

b. Strains: The discovery of rat virus (RV) by Kilham (sometimes referred to as "KRV" or Kilham's rat virus, not to be confused with "K" virus, a papovavirus of mice also isolated by Kilham) was the first viral isolation in the family Parvoviridae, and RV is the type species of the genus *Parvovirus*. All strains of RV cross-react serologically, and therefore strain differentiation is made primarily on the ability to produce a variety of pathological effects in rats and hamsters, (e.g., all except strain L-S are highly pathogenic for hamsters; KRV strain 308 crosses the placenta in rats, while KRV strain 171 does not), host range in cell culture, and the ability to agglutinate red blood cells from different animal species. A second antigenic type of rat parvovirus was isolated by Toolan and was desginated H-1. Although both RV and H-1 are antigenically distinct by the hemagglutination-inhibition (HAI) test, they do share either a common or cross-reacting antigen(s) as demonstrated in the fluorescent antibody (FA) test. Both viruses also cause a natural disease in laboratory and wild rats, induce developmental abnormalities in suckling hamsters, and are biochemically and physically similar.

c. Cultivation: Rat parvoviruses are "nondefective" and therefore do not require a "helper" virus to replicate. Inoculation of subconfluent primary rat embryo cell culture is the best in vitro method for propagating rat parvoviruses. However, the source of rats used to prepare the rat embryo monolayers must be prescreened and shown to be free of these agents. They are present in many commercial colonies and are often teratogenic, potentially infecting developing fetuses and resulting in the contamination of embryonic cell cultures, which will deteriorate with classical rat parvovirus CPE. Several established cell lines have been used also to grow various strains of rat parvoviruses such as the SV40-transformed newborn human kidney cell culture designated 324K, NRK, rat nephroma, BHK21, BHK35, and AT cells.

d. Stability: Rat parvoviruses are very stable. They are unaffected within a pH range of 2 to 11, remain infective after exposure to 80°C for over an hour, and are not inactivated by organic solvents such as alcohol, ether or choloroform. They have even been shown to remain infective after being maintained at room temperature for over a year.

e. Inactivation: Aside from a report that ultraviolet light will inactivate rat virus, additional information is lacking. Nevertheless, the EPA has registered several "cold sterilants" (utilizing either chlorine dioxide or gluteraldehyde as the active ingredient) which have been shown to inactivate feline and mouse parvoviruses. Consequently, they may also be able to inactivate rat parvoviruses.

2. Characteristics of Infections

a. Animals: Although only wild and laboratory rats have been shown to undergo natural infection, rat virus has a wide host range when administered experimentally, as demonstrated by its ability to infect hamsters, mice, and newborn cats, while the H-1 strain has an even wider host range, infecting rhesus monkeys and human volunteers.

b. Geographic distribution: Worldwide.

c. Means of spread: Certain strains of rat virus and H-1 are capable of both horizontal and vertical (transplacental) transmission. Horizontal transmission is most likely to occur by a fecal-oral cycle, but an oral-oral spread has also been proposed. Rat virus has been isolated from the feces and milk of infected rats. Although it has not yet been proven in controlled studies, circumstantial evidence to date indicates that rat parvoviruses cause persistent infections.

d. Morbidity and mortality: Although rats of all ages are susceptible to infection by rat virus, clinically overt disease characterized by runting, ataxia, and jaundice is only manifested in animals under 10 days of age. Nevertheless, adult rats exposed to conditions of stress will develop clinical signs of disease ranging from paralysis to ruffled fur coats, cyanotic scrota, and dehydration. When RV enters a breeding colony of rats, a loss of production is witnessed initially, due to numerous intrauterine resorptions and neonatal deaths. Production indices soon return to normal, however, as maternal antibody develops resulting in protection of the newborn rats until weaning, after which time the infection causes only subclinical disease. On the contrary, type H-1 is not known to cause any clinically overt disease following natural infection, even after initiating an epizootic in a naive breeding colony of rats.

e. Prevalence: Serological surveys have demonstrated that rat parvoviruses are present in numerous commercial and institutional facilities. Within any given colony, as many as 85 percent of the rats will be antibody positive in the HAI test.

f. Pathology: Rat parvoviruses, like other parvoviruses, have a predilection for rapidly dividing cells, especially in the late S phase of growth. In rats infected with RV prior to the 4th day of life, intranuclear inclusions are present in the actively mitotic cells composing the external germinal layer of the cerebellum. This leads to necrosis, thereby preventing the normal development of the cerebellum and resulting in granuloprival cerebellar hypoplasia (Jacoby, Bhatt, and Jonas 1979). This condition is expressed clinically as ataxia. Rat virus also causes a severe hepatitis, and some investigators consider the liver as the primary target organ. Affected animals are jaundiced, and intranuclear inclusions are seen predominantly in hepatocytes, which eventually die forming foci of necrosis. Peliosis hepatis is a possible sequela. The HER strain of rat virus causes a hemorrhagic encephalopathy in suckling rats under 10 days of age and in immunosuppressed adult rats. The hemorrhages result from the viral destruction of the endothelial lining of small blood vessels and eventually develop into small hemorrhagic foci predominantly in the white matter of the brain and spinal cord. Hemorrhagic lesions accompanied with thrombosis and necrosis have also been witnessed in the epididymis and testes of weanling rats during a natural epizootic, resulting in cyanotic scrota.

g. Diagnosis: A preliminary diagnosis of rat parvovirus disease can be made upon observation of typical gross and histopathological lesions and/or the presence of antibody in the hemagglutination-inhibition (HAI) test using guinea pig erythrocytes. Very few false positive results have been witnessed in the HAI test, and sera from infected rats consistently demonstrate high titered antibody. As with all infectious diseases, however, a definitive diagnosis can only be made after isolation and characterization of the etiological agent. The most dependable method for isolating rat parvoviruses is the inoculation of subconfluent primary rat embryo cell cultures derived from rats pretested and shown to be free of rat parvovirus infection. A rat antibody production (RAP) test is also a very sensitive means of detecting the presence of adventitious murine viruses of the rat, including the parvoviruses.

h. Interference with research: Because of the ability of rat parvoviruses to cross the placenta of rats and cause developmental abnormalities and fetal deaths, they are a major threat to teratological studies conducted in this species. These viruses can also be isolated from organs of infected rats in the presence of high titered circulating antibody and therefore will often contaminate transplanted tumors passed in infected rats. Once a tumor has been

infected with a rat parvovirus, it can persistently infect the tumor cells and thereby serve as a potential contaminant for recipient rats that are free of infection. For this reason, tumors should be tested for the presence of rat parvoviruses in the RAP test before they are introduced into a research facility that is known to be free of rat parvoviruses. If rat virus is already present in a group of rats, either environmental or experimental stress can precipitate clinically overt disease and cause deaths even in adults.

3. Control/Prevention

a. Special considerations: Since evidence to date indicates that rat parvoviruses cause persistent infections in rats, the only way to eliminate them from a facility is to eliminate the entire rat population and restock with uninfected animals following disinfection of the premises.

b. Effectiveness of vaccines: Unlike feline panleukopenia, there are no vaccines available for rat parvoviral diseases.

c. Effectiveness of hysterectomy derivation: Many strains of rat parvoviruses are capable of transplacental transmission, as many virologists preparing rat embryo cell cultures have discovered. However, since some strains of virus do not cross the placenta, one can hysterectomy-derive rats, hold them in isolators until adulthood, and then test them for infection by assaying their sera for HAI activity.

4. Test Procedures

a. Isolation: Rat parvoviruses can be isolated in cell culture and/or naive rats (RAP), both of which must be pretested to be shown free of adventitious murine viruses. The most suitable inocula are homogenates of bone marrow, lymph nodes, or spleen prepared from affected animals. If the virus is present in these organs, characteristic rat parvovirus cytopathogenic effect (CPE) will often be observed in cell culture and seroconversion in the HAI test will be noted in the RAP test animals. If no CPE develops in cell culture, the monolayers should be tested for the presence of viral antigen in the hemadsorption test while the cell culture fluids should be tested for HA activity using guinea pig erythrocytes.

b. Serology: FA, serum neutralization, complement-fixation, and HAI antibody assays have been described for RV and H-1. The HAI is the test of choice since it is the most convenient, can be performed within a few hours, and is highly dependable, yielding a very small percentage of either false positive or false negative results.

c. Histopathology: A description of the histopathology of rat parvoviruses is combined with the gross pathology in the "**Pathology**" section above.

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Sendai Virus

Kosaku Fujiwara and Joseph E. Wagner

1. **Agent: Sendai virus (Family: Paramyxoviridae; Genus: *Paramyxovirus*).**

   a. Morphology: Approximately spherical RNA virus 100 to 200 nm in diameter with a relatively rigid nucleocapsid enclosed in a lipid-containing envelope studded with projections 8 to 15 nm long and 2 to 4 nm wide.

   b. Cultivation: Propagation readily achieved in fertile eggs, most successfully by intra-amniotic inoculation. Growth in the allantoic cavity is best on passage. Assayed also by plaque formation in human, bovine, and simian cell cultures.

   c. Strain: Most isolates are similar in pathogenicity for mice and antigenically indistinguishable.

   d. Inactivation: Sensitive to heat, ether, chloroform, and formaldehyde. Inactivated at 56°C for 2 hr., but stable at −20°C.

2. **Characteristics of infection**

   a. Animals: Sendai virus causes epizootics with high mortality in young mice, especially in highly susceptible strains such as DBA/2J and 129/J. Many strains have clinically inapparent infections. In large breeding colonies, inapparent infections occur commonly in weanling animals that are only partially protected by diminishing maternal antibody. Normally mild infections in adult mice or rats may be severe in cases of pulmonary co-infection with other microorganisms. Antibody to Sendai virus is often found in guinea pigs, cotton rats, and hamsters, but outbreaks have not been well documented.

   b. Humans: Not generally accepted that Sendai virus causes human infection.

   c. Geographic distribution: Worldwide, in particular in Japan and the U.S.A.

   d. Means of spread: Transmission is mainly by direct contact through respiratory-oral routes. The virus may spread also from cage to cage and room to room by fomites and airborne droplets of nasal discharge.

   e. Morbidity and mortality: Occurrence of clinical disease and mortality in mice and rats depends upon the age, strain of animal affected, immunopotency, and the presence of other potential pathogens in the environment. Suckling and recently weaned mice are most susceptible and have high mortality. Although it is believed that co-infections with organisms such as *Mycoplasma pulmonis, Pasteurella pneumontropica*, or *Corynebacterium kutscheri* often result in severe pneumonia with high mortality, this has been poorly documented. Nude mice suffer from a subacute or chronic form of disease with persistent virus infection.

   f. Prevalence: Sendai virus may persist for long periods in large breeder colonies, even after most of the breeders have survived infection and have antibody. In such colonies there is continuous supply of young animals that become susceptible at the time of waning of maternal antibody. The virus spreads and persists among these 4-to-6-week-old animals. In contrast, in small colonies the virus may disappear after nearly all individuals have been infected.

   g. Pathology: The lesions of acute Sendai Virus infection are characterized by catarrhal bronchitis with necrosis and desquamation of bronchial epithelium. Epithelial lysis and denudation of air passages may extend into the alveolae. In the subacute and recovery phases, reparative epithelial proliferation may be found in the affected regions, often producing patchy squamous metaplasia. Reepithelialization of alveolae by cuboidal

epithelium often results in focal areas of adenomatoid appearance which have been described as "alveolar bronchialization." In chronic infections of nude mice, squamous metaplasia and hyperplaisa of bronchial epithelium are sometimes remarkable.

h. Diagnosis: Acute illness can be clinically diagnosed by noisy respirations, ruffled hair, and weight loss. Virus-specific immunofluorescence in the bronchial epithelium is useful on stamp preparations or sections from acute cases. Serology is effective for diagnosis in animals subclinically infected or those that survived acute infection.

i. Interference with research: Sendai virus infection is an important cause of interference with rodent production and research. Breeder or user rodent colonies generally do not stay free from Sendai virus for long time periods. Interference with research usually involves immunological and respiratory system studies. There is little information about the duration of such interference. Both acute and subclinical illness may cause data variations. Inapparent infections in breeder colonies may be a source of virus infection to all subsequent users.

3. Control/prevention:

a. Special considerations: Usually eradication is achieved by killing the animals, disinfecting the facilities, and repopulating with Sendai-free animals. In a confined facility it is also possible to eliminate the infection over a period of several months by halting breeding activities and preventing introduction of susceptible animals while allowing the disease to run its course. Immune survivors are then used as breeders to repopulate the colony.

b. Effectiveness of vaccines: Vaccine may be used to protect small nucleus colonies from infection. It is not recommended for large production colonies since it would be impossible to detect spontaneous infections by seromonitoring. Vaccination may be used also in long-term experiments in which a strict barrier system is difficult to operate.

c. Effectiveness of hysterectomy derivation: Since there has been no evidence of transplacental infection with Sendai virus, which is strictly pneumotropic, hysterectomy derivation should be effective for establishing new Sendai-free colonies.

4. Test procedures

a. Isolation: Sendai virus can be isolated in young mice by intranasal inoculation and in chick embryos by inoculation of the amniotic or allantoic cavity.

b. Serology: Both CF and HI tests are sensitive enough for screening large numbers of sera. Since no great difference is recognized between CF and HI titers of rat and mouse sera and since some mouse sera may give nonspecific positive results in HI, CF may be preferred for rodent sera. ELISA as well as IFA appear to be more sensitive than the CF and HI tests, but they are still not widely used for screening.

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II. Bacteria and Mycoplasma

B. piliformis

Kosaku Fujiwara and James R. Ganaway

1. Agent: *Bacillus piliformis* (Tyzzer) (Classification: Unknown). Disease induced: Tyzzer's disease.

a. Morphology: Gram-negative uniform rod-shaped bacillus, 8 to 10 μm long by 0.5 μm wide during logarithmic growth with tendency toward pleomorphism (monilial swellings, variations in length, and affinity for basic aniline dyes) during altered metabolism. Terminal endospores are formed. Motile by peritrichous flagella. Readily stained with basic aniline dyes. PAS-positive. Silver impregnation techniques superior for demonstration in tissue sections.

b. Cultivation: No growth in cell-free media: obligate intracellular parasite. Successful propagation in embryonated hen's eggs inoculated via the yolk-sac route, or in primary hepatocyte cultures of chick embryo or mouse.

c. Strains: Isolations have been made in mice or embryonated eggs from several species of animals ill or recently dead with Tyzzer's disease including mouse, rat, hamster, gerbil, domestic cat, rabbit, and horse foal. They appear to be antigenically related but not identical. Further study is needed.

d. Stability: The vegetative bacillus outside the viable host cell is unstable and rapidly looses virulence except when stored at -70°C. Spores withstand repeated cycles of freeze and thaw as well as 60°C for 1 hour but are inactivated at 80°C for 30 minutes.

e. Inactivation: Due to the very unstable nature of the vegetative bacillus outside the viable host cell, inactivation studies have not been performed. The spores are resistant to ethanol, phenolic germicidal detergents, and quarternary ammonium compounds but are inactivated by formalin, iodophor, peracetatic acid, or sodium hypochlorite. The latter as a 0.3 percent solution is recommended.

2. Characteristics of infection

a. Animals: Tyzzer's disease affects a wide variety of animals including mice, rats, hamsters, gerbils, guinea pigs, laboratory rabbits, cats, dogs, cottontail rabbits, muskrats, coyotes, foxes, snowshoe hares, rhesus monkeys, and horses.

b. Humans: No human case has been reported, although a spontaneous case in a rhesus monkey was recorded.

c. Geographic distribution: Reported in many countries worldwide.

d. Means of spread: As the vegetative bacillus is unstable outside the viable host cell, the most probable means of spread is by ingestion of spores which are shed in feces.

e. Morbidity and mortality: Epizootics associated with high mortality occur in gerbils, hamsters, muskrats, and rabbits. Immunosuppression (e.g., cortisone administration or x-irradiatin) can induce clinical and often fatal infection during otherwise inapparent infections.

f. Prevalence: In stocks where the disease has been diagnosed, the prevalence is probably high unless specific measures to control fecal-oral spread are taken. Limited serologic studies using immunofluorescent antibody technique and complement fixation indicate that antibody to *B. piliformis* is prevalent in mouse, rat, and rabbit colonies. Antibody has also been detected in chickens, pigs, horses, cats, dogs, humans, and several species of wild rodents.

g. Pathology: Lesions of Tyzzer's disease are characterized by necrosis of selected cells and the demonstration of typical randomly arranged rod-shaped bacilli in the cytoplasm of apparently viable cells at the border of the necrotic areas. Varying degrees of cecocolitis, hepatitis, and myocarditis may be seen. Enteric lesions (with demonstration of

the bacilli in epithelial cells and smooth muscle cells of the muscularis mucosa) tend to be severe in certain species such as rabbit, hamster, and gerbil. The most frequently encountered lesions in all species of affected animals is focal hepatic necrosis. Neutrophils and mononuclear cells infiltrate affected areas, and the bacilli are absent in healing lesions. The method of choice for demonstrating the bacilli in all affected tissues is the silver strain (e.g., Warthin Starry).

h. Diagnosis: The demonstration of the bacillary agent in the cytoplasm of cells at the border of necrotic areas forms the basis for diagnosing acute fatal Tyzzer's disease. Identification of the agent in tissue can be confirmed with fluorescent antibody techniques. Sero diagnosis of inapparent infections can be accomplished using complement-fixation (limited value for survey due to low titers in mice) or indirect fluorescent antibody. Isolation using freshly prepared liver suspension is possible in weanling mice (intracerebrally or intravenously with cortisone) or in embryonated eggs inoculated via the yolk-sac route.

i. Interference with research: Epizootics associated with high mortality have been observed in certain species of animals, especially at weaning age, when the animals are stressed by crowding, movements, poor sanitation, and cortisone administration or irradiation. The interpretation of toxicology studies involving the liver could be influenced by intercurrent Tyzzer's disease.

3. Control/prevention

a. Special consideration: Infective spores are shed in the feces. Good sanitation and frequent changes of cage and bedding will help control the build-up of spores in the environment (see paragraph 1.e. above for inactivation of spores).

b. Effectiveness of vaccines: An effective vaccine is not available. Experimentally, protection from otherwise fatal challenge can be induced by multiple injections in mice of a formalin-treated liver emulsion prepared from diseased mouse liver. This procedure did not completely prevent induction of lesions in the liver of challenged mice, however (see paragraph 1.c. for antigenicity of various strains).

c. Effectiveness of hysterectomy derivation: Infection of the uterus or conceptus has not been observed in naturally-occurring Tyzzer's disease. However, when pregnant mice are challenged intravenously *B. piliformis* can be detected in the endometrium and fetal liver, indicating that this agent can cross the placental barrier. Also, antibody has been demonstrated (indirect immunofluorescence) in the sera of barrier-maintained colonies of mice, rats, and rabbits.

4. Test procedures

a. Isolation: Few isolations have been made, presumably because of the unstable nature of the vegetative phase outside the host cell and the encounter of other intestinal microflora in the liver of acute cases of fatal disease. The latter is particularly a problem when attempting isolation in embryonated eggs inoculated via the yolk-sac route or cell cultures. The probability of successful isolation is increased when cortisone-treated (2.5 mg s.c.) weanling mice are inoculated intravenously with freshly prepared liver suspension from severe acute cases and blind-passaged at 3-to-4-day intervals.

b. Serology: CF test is sensitive enough to permit screening of rat colonies but not mouse colonies. Two procedures useful for determination of serotypes are CF or gel precipitation with infected mouse liver antigen and mouse protection test by serum treatment made before inoculation. Antigens are prepared from infected mouse liver emulsion. It is allowed to stand at 4°C for 2 or 3 days for bacteriolysis, and after treating with ether, the water phase is used as antigen. The indirect fluorescent antibody technique is the most sensitive serologic procedure presently available.

c. Histopathology: Characteristic lesions are described in the sections **"Pathology"** and **"Diagnosis"** above.

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Citrobacter freudii

Stephen W. Barthold and Ruth L. Adams

1. **Agent: *Citrobacter freundii* (Kingdom: Procaryotae; Division: Gracilicutes; Class: Scotobacteria; Order: not known; Family: Enterobacteriaceae; Genus: *Citrobacter*). Disease induced: Transmissible murine colonic hyperplasia.**

   a. Morphology: The mouse pathogens are variants of *C. freundii*. They are nonmotile, nonencapsulated rods, measuring 1.5-2.0 μm. These isolates lack flagellae and fimbriae both in vitro and in vivo. In contrast, most members of the genus *Citrobacter* are motile with peritrichous flagellae. There are presently no selective media available which accurately distinguish *C. freundii* from *Escherichia coli* without further biochemical testing.

   b. Cultivation: Most *Citrobacters* use citrate as a sole carbon source, and lactose fermentation is absent or delayed. In contrast, mouse pathogenic isolates of *C. freundii* usually do not utilize citrate and readily ferment lactose. However, variable reaction to citrate has been reported with one mouse *C. freundii* isolate, but this may have been due to inconsistencies in inoculum size. Unlike typical *C. freundii*, mouse pathogenic isolates are nonmotile, produce little or no H_2S, and utilize malonate. Lactose, glucose, mannitol, sorbitol, rhamnose, maltose, xylose, amygdalin, and arabinose are regularly fermented, but not inositol, sucrose, or melibiose. Lysine is not decarboxylated. Indole is not produced. An effective means of definitive identification can be achieved with API 20 enterobacteriaceae strips (Analytab Products, Inc., Plainview, NY). The identifying profile for the most extensively studied mouse *C. freundii* variant is 1104513. This strain is differentiated from *E. coli* by its lack of motility, lack of indole production, and utilization of malonate.

   c. Strains: A large number of *Citrobacter* spp. and *Citrobacter freundii* strains can be isolated from a wide range of animal species. Most *Citrobacters* are nonpathogenic, although some are opportunistic and occasionally primary pathogens. Disease in mice is caused by a single or similar atypical variants of *C. freundii*. Relatedness of mouse isolates has been tested on a biochemical, but not antigenic or DNA homology basis. *C. freundii* ANL, *C. freundii* (Ediger) and *C. freundii* (4280) have been isolated from mice with similar enteric disease. These strains resemble one another, but minor biochemical differences exist. *C. freundii* (4280) is the most well characterized strain and seems to be biochemically identical to strains isolated from diseased mice in a number of institutions in the last several years.

   d. Stability: Mouse *C. freundii* isolates are resistant to the penicillins and cephalosporins but are sensitive to the aminoglycosides and tetracyclines. Survives lyophilization and storage at -70°C indefinitely.

   e. Inactivation: Thermolabile: inactivated by ethylene oxide as well as moist heat sterilization.

2. **Characterization of Infection**

   a. Animals: The mouse is the only species known to be naturally or experimentally susceptible to enteropathogenic variants of *C. freundii*. Atypical *C. freundii* has only been isolated from mice with enteric disease and appears to produce disease only in mice. Unrelated, nonpathogenic *C. freundii* can be isolated from the bowel of rodents, particularly hamsters and guinea pigs. In an experimental study, *Citrobacter* isolates from other mammalian and reptilian species did not colonize the mouse bowel and the mouse *C. freundii* variant did not colonize the rat or hamster bowel following oral inoculation. Mice of all strains and ages seem to be susceptible to *C. freundii*, but clinical signs and lesion severity vary.

b. Predisposing factors: A number of factors, including genetic background, composition of diet, and age have been shown to significantly modify the severity of lesions induced by *C. freundii* in mice and, thus, clinical signs of disease. Mice become infected with *C. freundii* and develop intestinal lesions regardless of these factors, however. Autochthonous intestinal microflora may provide a degree of resistance to *C. freundii* as is seen with other intestinal pathogens, but this has not been examined. Prevalence of infection is higher in mouse colonies maintained with marginal husbandry or in which there is cage-to-cage transfer or handling. Experimental stress has been reported to precipitate natural disease.

c. Geographic distribution: *C. freundii*-associated enteric disease in mice has been reported in the United States and Europe. Distribution is probably more widespread, but infection is often unrecognized.

d. Means of spread: Pathogenic mouse *C. freundii* appears to spread slowly under natural and experimental conditions, probably by direct contact. Orofecal contamination is the likely means of spread. Husbandry practices may enhance or limit spread between cages. The source of infection in different documented outbreaks has never been determined.

e. Morbidity and mortality: Virtually all mice are susceptible to pathogenic *C. freundii* regardless of strain or age. The severity of lesions, however, is greatly influenced by these factors, as well as undetermined components in the diet. Morbidity and mortality are usually low in an affected population, but may assume significant levels depending upon interaction of husbandry conditions, genetics, age, and diet. Under natural conditions, mortality or runting is usually seen in weaning-age mice. Adult animals can have enteric lesions but do not often show clinical disease. A variable incidence of rectal prolapse in mice of all ages is often indicative of infection.

f. Prevalence rate: Prevalence of detectable infection is usually low, even in the course of a disease outbreak. Spread of *C. freundii* is slow, and infection rate is therefore usually low. Furthermore, there appears to be no carrier state. Pathogenic *C. freundii* is present in affected mice only in the early phase of the disease and is often absent when clinical signs are manifest. The organism and lesions may be restricted to the distal bowel. Because of these factors, as well as the frequency of subclinical infection, *C. freundii* is often difficult to diagnose and often goes unrecognized. The prevalence of infection among different institutions is unknown, but apparently not common.

g. Pathology: The pathology descriptions in the literature which are associated with *C. freundii* in mice vary, but this is a reflection of the stage of disease examined in different mice rather than true differences. The pathogenesis of *C. freundii* has been extensively examined at all stages of infection in the mouse. Following oral inoculation, *C. freundii* is initially isolated from small intestine, but soon is restricted to the large bowel. Within 3-4 days, myriads of *C. freundii* become intimately attached to the surface mucosal epithelial cells of the descending colon. The transverse and ascending colons and cecum are less frequently involved. Bacterial attachment causes distortion and loss of microvilli. Occasionally, colonies of *C. freundii* can be discernable within necrotic areas. During this phase, *C. freundii* is the only aerobic bacterium in the large bowel. In response to changes in the surface mucosa, crypt base cells become mitotically active. As they proliferate, crypts elongate and become populated by rapidly migrating, mitotically active immature epithelial cells. As these cells emerge from crypts, they push aside *C. freundii*-infected cells on the surface, which are eventually extruded. Reinfection of newly arising epithelium does not occur. When the hyperplastic response has reached its zenith at 2-3 weeks, *C. freundii* is no longer present. The hyperplastic response can be accompanied by inflammation and erosion. Certain mouse strains and juvenile mice develop severe inflammatory changes. Clinical signs, including pasty dark feces, dehydration, rectal prolapse, and mortality occur on or after the peak of the hyperplastic response and appear more related to the degree of inflammation than of hyperplasia. With the loss of *C. freundii*, regression of

hyperplasia takes place. Mitotic and migration rates slow and cells undergo maturation, often with excessive mucinous differentiation. By 1.5-2 months, the mucosa returns to normal, except in mice with rectal prolapse, which may be persistant. Mice are refractory to reinfection, but are experimentally susceptible to reinfection after a few months.

h. Diagnosis: Definitive diagnosis is attained by demonstration of typical lesions in the descending colon and other levels of large bowel and isolation of the causative agent. This is often difficult to achieve in a small sample of mice. Gross lesions can be undetectable to the inexperienced eye, and characteristic histopathology is often restricted to the distal 1 cm of bowel, which may not be routinely sampled at necropsy. Furthermore, *C. freundii* may no longer be present in the bowel when clinical signs become apparent. Pathogenic *C. freundii* has never been found to be a normal inhabitant of the mouse bowel, and infection has always been associated with lesions. Other nonpathogenic strains of *Citrobacter* can inhabit the mouse bowel and must be differentiated from the pathogenic strain. Routine surveillance of mouse colonies for *C. freundii* by fecal culture is not advocated. The likelihood of random isolation is low, and there is no current economical method to differentiate pathogenic *C. freundii* from the far more common *E. coli*. Detection in a mouse colony is most effectively accomplished by implementation of a sound diagnostic capability to complement a surveillance program for other agents.

i. Interference with research: Aside from its obvious effect on the health of mice, *C. freundii*-induced hyperplasia can significantly alter the early course of large bowel chemical carcinogenesis by promoting the early evolution of preneoplastic focal atypia in high incidence with otherwise subthreshold doses of carcinogen. The hyperplastic lesion itself has been confused with neoplasia, and its cytokinetics share several common features with neoplasia or preneoplasia. Experimental stress can precipitate more severe disease among infected mice.

3. Control/prevention

a. Special considerations: Control of *C. freundii* is based on an understanding of the infection. The organism spreads slowly by orofecal contact. Thus, attention to sanitation and handling of mice can greatly reduce the prevalence. There is no known carrier state, so infected animals can be effectively prevented and eliminated by antibiotics. Neomycin sulfate (2 mg active ingredient/ml) and tetracycline hydrochloride (1 mg/ml) can be administered in the drinking water, and both have been shown to be effective against pathogenic *C. freundii* in mice. Pathogenic *C. freundii* is rarely seen in mouse colonies where scrupulous attention is paid to sanitation and animal handling techniques.

b. Effectiveness of vaccines: Not necessary and probably ineffective. Naturally infected mice become susceptible within a few months after recovery.

c. Effectiveness of hysterectomy derivation: Although *C. freundii* can be eliminated by a number of means, hysterectomy derivation or repopulation with clean stock is the most effective means of eliminating the infection in a mouse colony.

4. Test Procedures

a. Isolation: *C. freundii* can be isolated from feces, large bowel contents, or bowel wall of mice during the initial phase of infection. It grows readily on common as well as selective media used in isolation of fecal pathogens. Mouse pathogenic *C. freundii* isolates are difficult to distinguish from *E. coli* on MacConkey agar with any degree of accuracy (see **"Cultivation"**).

b. Serology: None available.

c. **Histopathology:** (see **"Pathology"** above). Mucosal hyperplasia is usually present in the descending colon. Other levels of intestine are less consistently affected. Thus, the pathologist should always examine the distal 1 cm of colon for lesions in mice with signs of intestinal disease. Lesions are characteristic but can be confused with mucosal hyperplasia caused by other pathogens, especially mouse hepatitis virus, *Salmonella*, and *Bacillus piliformis*.

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Corynebacterium kutscheri

Stephen H. Weisbroth

1. Agent: Corynebacterium kutscheri (Kingdom: Procaryotae; Division: Firmicutes; Class: not known; Order: not known; Family: Coryneform group; Genus: *Corynebacterium*). Disease induced: Corynebacteriosis.

a. Morphology: Irregularly staining, slender Gram positive diphtheroid rods with metachromatic granules. Smears and sections from infected tissues stained with Giemsa, Gram, Brown and Brenn, or Gram-Weigert show irregular palisades or "Chinese letter" rod aggregates.

b. Cultivation: Tissue aspirates, exudates, and organ or tissue triturates are cultured directly on 5 percent sheep blood agar (trypticase soy base) for isolation from clinical material. Colonies are 1-2 mm in size after 24 hour incubation at 37°C, circular, entire, dome-shaped, greyish to yellowish white in color, smooth, and nonhemolytic. Isolates of *C. kutscheri* are facultatively aerobic, nonmotile, oxidase negative, catalase positive, MR/VP:(−)/(−), and do not liquify gelatin or produce indole. They do reduce nitrate, are urease positive, and ferment glucose, sucrose, maltose, salicin, fructose, and mannose to produce acid. They do not ferment lactose, mannitol, or dulcitol, nor do they grow on MacConkey agar. Fermentation of xylose and H_2S production are variable. May be propagated on trypticase soy agar or in trypticase soy broth.

c. Type strain: ATCC 15677, NCTC 949. Type strains with arabinose in cell wall have buoyant DNA density of 1.717 and G-C content of DNA of 58.2. Older references to presumed avirulent variants not sustained by more recent research. In the current view, although minor serologic differences may exist in homology between isolates from various host species, the great majority of *C. kutscheri* isolates form a culturally, morphologically, ecologically, and serologically homogeneous group.

d. Stability: Although *C. kutscheri* does not form spores, it should be regarded as quite stable at room temperature in exudates, urine, and feces. Stable L-forms have not been demonstrated. Antibiograms have been published with profiles of antibiotic sensitivity and resistance. Should be regarded as requiring heat exposure of at least 180°F to inactivate. Stable for long periods when lyophilized or frozen at -70°C.

2. Characteristics of Infection

a. Animals: Naturally occurring infections of *C. kutscheri* appear to be limited to the rat (*Rattus norvegicus*) and mouse (*Mus musculus*) although the organism has been isolated from guinea pig. Infections in rats and mice occur sporadically as abscesses of superficial tissues. Epizootic rat infections occur as pulmonary embolization, whereas mouse infections have more typically resulted in septic embolization of the joints, liver, and kidney.

b. Predisposing factor: Although *C. kutscheri* has been encountered as a primary pathogen in unprovoked epizootics in both rats and mice, more commonly, in both host species, inapparent or latent infections have been unmasked by experimental procedures that lower host resistance or impair immunocompetence. Such treatments have included vitamin deficiency, viral (ectromelia) and bacterial (*Salmonella*) infections, ionizing radiation, and steroid (e.g., cortisone and hydrocortisone) administration. Heritably conditioned resistance (and susceptibility) has been demonstrated among various rat and mouse strains.

c. Geographic distribution: Worldwide

d. Means of spread: Not known with certainty, but the organism may be isolated from oropharynx and nasopharynx of infected rats and mice. Urine and feces of infected mice should be regarded as infective, and by inference, contact with soiled bedding from such animals. Vertical transmission (prenatal) has been experimentally demonstrated. Because of the chronicity of clinically silent, latent infections, the actual origin of epizootics, when triggered, is often obscure.

e. Morbidity and mortality: Both morbidity and mortality are extremely variable and depend, for expression, on the interaction of husbandry, heritability, and resistance-lowering procedures. Infections may be entirely latent and clinically silent. States of resistance are indicated by abscesses of superficial tissues. Suddenly induced susceptibility, as by treatments indicated above as predisposing factors, is manifested by septicemic embolization of organs with capillary nets. Epizootics rarely involve more than 10-20 percent, and more commonly, 2-5 percent of the population at risk. The animals expressing morbidity and mortality should be regarded as representing only a small fraction of the actual (latently) infected population. There is no association with any particular age or sex group.

f. Prevalence: With recent common availability of hysterectomy-derived, barrier-maintained rodent stocks, and under elevated husbandry conditions that commonly prevail, *C. kutscheri* infections are increasingly infrequent.

g. Pathology: Latent, clinically silent infections may not be associated with tissue pathology, and *C. kutscheri* may be isolated from nonlesioned tissues. States of host resistance are pathologically manifested by abscessation of superficial tissues, e.g., pododermatitis, preputial gland abscess, and abscessation of superficial lymph nodes, and by middle ear infection (tympanitis). States of susceptibility are indicated by relative absence of superficial containment; i.e., infection results in hematogenous dissemination (embolization) and microabscessation of various internal organs. Even the predominatly pulmonary pattern of infection in the rat is interstitial rather than bronchiolar, and thought to follow hematogenous dissemination. Centrally, such lesions originate as foci of coagulative necrosis, but become liquifactive as acute inflammatory cells infiltrate. In nonfatal cases, the lesions may mature to become inspissated or caseous. Granuloma formation, as in pseudotuberculosis, is not recognized in rodent infections. Organisms may be indistinct with H&E and are not acid-fast, but may invariably be demonstrated in sections with Giemsa or Gram stains.

h. Diagnosis: Presumptive diagnosis may be established by the demonstration of typical palisading Gram-positive rods in "Chinese letter" arrangement from smears of lesioned tissue. Definitive diagnosis requires isolation and characterization of isolate organisms according to the criteria above under **"Cultivation."** Histopathologic analysis may be strongly suggestive when used retrospectively in the absence of bacterial isolation. Serodiagnosis is being used with increasing frequency, but the effectiveness (i.e., sensitivity) of current serologic methods to detect latent infection is not established.

i. Interference with research: Although the prevalence of *C. kutscheri* diagnoses has declined in recent years, this organism has a long and well deserved reputation for emergence as a clinical entity during experimental procedures in fields as diverse as oncology, radiation biology, nutrition, toxicology, and pharmacology. Interference usually takes the form of premature termination of the project due to morbidity and mortality, rather than to more subtle nuances of data interpretation.

3. Control and Prevention

a. Special considerations: The incidence of infection in colonies with morbidity is usually unknown, but should be assumed to be much larger than indicated by the actual number of animals with signs. Treatment with antibiotics may effectively suppress systemic infection and

reinduce latency, but common experience has been that such treatments are withdrawn, clinical reversion to overt susceptibility is resumed ntibiotic treatment does not eliminate the infection.

b. Vaccines: No information has been published on vaccine attempts.

c. Effectiveness of hysterectomy derivation: Although vertical transmission of *C. kutscheri* has been experimentally documented, intrauterine infection of rats and mice under conditions of natural infection has not. Derivation by hysterectomy and maintenance within barriers should be regarded as effective and most likely responsible for the decline of prevalence of this organism.

4. Test Procedures

a. Isolation: Exudates from abscess or from middle ear should be collected aseptically and plated directly on 5 percent sheep blood agar (trypticase soy base). Similarly, sterile saline or water aspirates used to wash nasoturbinates, the tracheobronchial tree, or the tympanic cavity may be plated directly. Organs and tissues should be triturated in a 1:1 (w/v) dilution with sterile saline or water using tissue grinders and the resultant triturate plated directly. Suspicious colonies, as determined by Gram stain morphology, should be characterized using criteria listed above under **"Cultivation."**

Colonies may be monitored for latent infection by using cortisone acetate injections of sample groups of rats or mice to provoke overt infection. The cortisone stress test is performed on groups of 10-20 rats or mice, depending on the size of the subject colony. Withholding food for the 24 hours preceding cortisone injection enhances the challenge. For mice, a 2.5 mg injection, and for rats, a 5.0 mg injection is given as a single subcutaneous dose on day 1. On day 14, surviving animals are euthanitized and representative tissues fixed for histologic preparations (liver, kidney, lung) and the same organs triturated for bacterial retrieval. Animals, if any, dying during the 14-day period should be processed for histologic and bacterial isolation as above for survivors.

b. Serology: At the present time, serodiagnosis may be regarded as established for detection of overtly infected rats and mice, or those surviving overt infection. The efficacy of serologic detection of latent carriers is uncertain at present. Tube agglutination, microtiter plate agglutination, and indirect fluorescent antibody (IFA) are in common use to detect antibodies to *C. kutscheri*, and are of about equal sensitivity. Agar gel immunodiffusion has been described as less sensitive than agglutination or IFA. ELISA has been recently described as a more sensitive method. Reagents for serologic immunoassay are not available commercially. The anamnestic response test was developed as a means of enhancing serologic detection in latent carriers expected to be very low titered or serologically negative. In this test, killed suspensions of *S. kutscheri* are injected IP, and the test animals bled on the 6th day. Antibody responses, if any, as determined by microtiter plate agglutination, are held to be anamnestic and reflective of previous experience (sensitization) with *C. kutscheri*.

c. Histopathology: Histologic sections stained with Giemsa and/or tissue Gram stains are quite useful and may be used to support diagnosis of overt infection, as well as provoked infections following steroid challenge. The invariable presence of microcolonies and "Chinese letter" aggregates of palisading rods approaches a pathognomonic finding.

d. Interpretive comments: Clinically overt *C. kutscheri* infection of laboratory rats and mice is not difficult to diagnose. The organism is morphologically distinct and retrieved with relative ease on ordinary media. Latent infection is more difficult and may be refractory to both diagnostic surveillance and eradication. For this reason, even a single overt case should cast suspicion on the entire group at risk. Even worse, the group at risk may act as a reservoir for spreading the infection to other colonies (i.e., rooms) within the facility. The only certain means of eradication is prompt liquidation of exposed groups, followed by vigorous sanitization of exposed environments.

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Mycoplasma pulmonis

James R. Ganaway

1. **Agent:** ***Mycoplasma pulmonis*** **(Division: Tenericutes; Class: Mollicutes; Order: Mycoplasmatales; Family: Mycoplasmataceae). Disease induced: Mycoplasmosis.**

   a. **Morphology:** Cells are pleomorphic and vary in shape from spherical or slightly ovoid (200-300 to slender filaments (2-5 um). Motile by gliding motion of bacilliform elements a constant rotation of coccoid structures. Cells lack a cell wall and are bounded by single triple-layered membrane (7.5-10 nm). Gram-negative.

   b. **Cultivation:** Enriched medium containing sterols required for growth. Optimal growth after 4 hours incubation at 37°C under aerobic conditions at pH 7.8. Unlike colonial gro of many other mycoplasma species, colonies are raised, the surface is coarsely granulated or vacuolated; there is little tendency to grow into the agar and thus d appear as typical "fried egg" colonies. Acid produced from glucose (fermentative Colonies on solid medium adsorb red blood cells from monkey, man (type 0), ra guinea pig and chicken; spermatozoa of man and bulls; various primary and continuous cell cultures.

   c. **Type strain:** Ash; PG34; ATCC 19612; AMRC-C61; NIH M-717-001-084. Isolates may lose virulence with repeated passage in vitro. Though minor differences have been demonstrated, the evidence suggests that strains are sufficiently similar to allow u one strain as antigen in serologic procedures.

   d. **Stability:** Relatively resistant to lysis by osmotic shock but very sensitive to lysis by surface agents and lypolytic agents. Resistant to 1,000-10,000 ug/ml of penicillin G, to 1,000-4,000 ug/ml of ampicillin, chloraxillin, methicillin or nafcillin, and to sulfonamides. Relatively resistant to erythromycin (minimum inhibitory concentr 40 ug/ml). Tolerates thallium acetate (1:2,000-1:4,000). Attempts to culture *M. pulmonis* from immediate environment (surface of inanimate objects, food, wate infected animals have failed. Survives freeze-drying and storage at -70°C.

   e. **Inactivation:** Thermolabile; no resting stage (spore); (see **"Stability"** above).

2. **Characteristics of Infection**

   a. **Animals:** Commonly associated with chronic respiratory disease in rats and mice; colonize female reproductive tract, sometimes but not always causing reproductive failure arthritis (Barden strain) produced experimentally following i.v. inoculation. Rare isolations from upper respiratory tract of rabbits and guinea pigs; relationship to disease not established. Although considerable variation has been reported in the response to experimental infection among species and among strains of the same species, all strains of mice and rats appear to be susceptible.

   b. **Predisposing factors:** High ammonia levels (caused by overcrowding, infrequent change of bedding/cle of cages, and use of filter caps on cages) increases severity of lesions in upper respiratory tract and frequency of lesions in lungs of rats. Indirect evidence sugg that concurrent infection with other agents (e.g., viruses and bacteria) would hav similar potentiating effect upon *M. pulmonis* respiratory infection in rats and mi

   c. **Geographic distribution:** Worldwide.

   d. **Means of spread:** Presumably by droplet via the upper respiratory tract. With good husbandry (fre changes of bedding/low levels of intracage ammonia) and absence of other respi pathogens, horizontal transmission is an unlikely event. Conversely, horizontal transmission, even from cage to cage, is predictably rapid (days/weeks) in an environment of high ammonia levels/other respiratory pathogen infections. Uteri

infection can result in vertical transmission to the young. This is the most likel explanation for introduction of *M. pulmonis* into germfree isolaters (following section) and, subsequently, into strict barrier colonies of mice and rats.

e. Morbidity and mortality: Under optimal husbandry conditions and absence of other respiratory pathoge morbidity and mortality are extremely low and may not be perceptible. Conve with poor husbandry and frequent infections with other respiratory pathogens, *pulmonis* may become an important factor in increasing morbidity and mortali which may begin at 6 to 8 weeks of age and become progressively severe with

f. Prevalence rate: See **"Morbidity and mortality"** for factors which influence prevalence rates in In poorly managed conventional colonies of mice and rats, the prevalence rate approach 100 percent. With optimal colony management incorporating cesarea derivation and strict barrer maintenance procedures, *M. pulmonis* can be elimi Once eliminated, mice and rats can be maintained free of *M. pulmonis* infectio clean conventional environment husbandry procedures provided infected anima not introduced into the colony.

g. Pathology: *M. pulmonis* colonizes the epithelial surface of the air passages and middle ear uterus, fallopian tubes and the synovium of distal extremities. In production co where infection is endemic, colonization of the nasal passages and middle ears during the nursing period. With unfavorable conditions (see **"Morbidity and mortality"** above), infection descends the respiratory tract to the trachea and b Chronic respiratory disease, with which *M. pulmonis* is commonly associated, is characterized by varying amounts of mucus and polymorphonuclear cell exuda air passages and lymphocytic and plasma cell infiltration of the submucosa. Lu abscesses, consolidation, and bronchiectasis are frequently seen in terminal case *pulmonis* can cause reproductive failure presumably by obstruction of the fallo tubes with purulent exudate or interruption of the uteroplacental attachments.

h. Diagnosis: Diagnosis depends upon isolation and identification. With experience and prop equipment, the immunofluorescence technique enables rapid diagnosis. Serology—complement fixation or enzyme-linked immunosorbent assay (ELISA useful to determine the status of a colony but may not detect early colonizatio upper respiratory passages in individual mice or rats due to insufficient stimula antibody production. A one-way cross-reaction has been reported between *M. pulmonis* and *M. arthritidis* using the ELISA technique.

i. Interference with research: There have been numerous instances in which experimental results have been unknowingly complicated by *M. pulmonis* infection, especially in respiratory di research, gerontologic studies, nutrition research, experimental toxicology, and behavioral research.

3. Control/Prevention

a. Special considerations: In colonies where the infection is well established, other respiratory pathogens present, and lung lesions are extensive, no effective means of eliminating the in is available (see **"Hysterectomy Derivation"** below). Antibiotic treatments (e.g. tetracyclines) may reduce morbidity and mortality but they do not elminate the infection. Shortly after cessation of antibiotic administration morbidity and mo increase.

b. Effectiveness of vaccines: Experimental studies suggest that a killed *M. pulmonis* vaccine will protect mic developing pneumonia when challenged intransally. High levels of IgG, whethe stimulated by natural infection or by killed vaccine, do not prevent colonizatio respiratory tract of the rat with *M. pulmonis*.

c. Effectiveness of hysterectomy dervivation: Cesarean derivation and barrier maintenance is the only effective means of elim *M. pulmonis* infection. This is true even though *M. pulmonis* can colonize the at full term of the pregnancy. Failure to determine the freedom of *M. pulmoni*

infection of the pups is the most logical explanation for "breaks" encountered in t
M. pulmonis-free status of barrier-maintained colonies. In barrier-maintained rats
are free of other respiratory pathogens, evidence suggests that infections (colonizat
with *M. pulmonis* may be self-limiting and disappear in subsequent generations in
closed colony.

4. Test Procedures

a. Isolation: The use of swabs/washings of various loci of the respiratory tract or uterus is preferred to preparing organ suspensions by grinding or homogenization, because the possible release of inhibitors present in the tissues. Though the probability of obtaining a positive culture is increased by culturing several sites, culture of a swa (Calgiswab) of the oropharynx of the intact rat is a reliable and sensitive method provides an effective means of determining infection in the live rat as well. With t aid of light narcosis (e.g., halothane) the rat ($\geq$ weanling age) is placed in a dorsa recumbent position; the tongue is grasped with a nonserated thumb forcep and extended, enabling the passage of the dry swab to the oropharynx. In so doing, mucous secretion present in the pharynx respiratorius (nasopharyngeal duct) is for into the pharynx by pressure of the swab on the palatum molle (soft palate). This therefore takes samples of secretions from the posterior nasopharyngeal duct whe eustachian tube enters the duct. The swab is gently rotated, removed, and washed 2.0 ml of nutrient broth (such as trypticase soy rather than mycoplasma broth containing inhibitors if determination of presence of other bacteria is desirable); b mycoplasma broth (0.1 ml washing/2-3 ml broth) and mycoplasma agar (one dro washing) are inoculated and incubated at 37°C. Plates are taped to prevent dryin examined under magnification (x45) after 96 hours incubation for typical *M. puln* colonies. In the absence of typical colonies on agar, the 96-hour broth enrichment plated (one drop) on agar and examined after incubation for 96 hours. Washings other areas of the respiratory tract, including middle ear (exudate readily obtained aspiration using a capillary pipette following passage through the ruptured tympa membrane) and from the uterus may be cultured in like manner. For description colonial growth see **"Cultivation"** above. Various staining procedures (e.g., Dien stain on unfixed colonies or Giemsa stain on Bouin's fixed impressions of colonie serologic procedures (e.g., metabolic inhibition, growth inhibition and fluorescent antibody), and red blood cell adsorption (see **"Cultivation"** above) are helpful in identification and specification. A very important aspect of attempted isolation of *pulmonis* from animals is *familiarization with the character of a known strain an testing the ability of each batch of mycoplasma medium (both broth and agar) to support the growth of known laboratory strains of M. pulmonis*. Mycoplasma m that will support *M. pulmonis* growth consists of mycoplasma base broth (seven pa pretested horse serum (two parts), 25 percent yeast extract (one part), penicillin G (1,000 units/ml final concentration), and thallium acetate (1:2,000 final concentra Add agarose at 1 percent final concentration for agar medium. If desired, glucos (0.5 percent) and phenol red may be added. However, caution should be exercise using a color change (acid from glucose) to indicate *M. pulmonis* growth in prim isolation attempts since metabolism of other cells in the washing (inoculum) can this color change.

b. Serology: The ELISA is a sensitive and rapid serologic procedure for surveying a populatio The complement fixation test can also be used but it is less sensitive than the EL Caution should be exercised in the interpretation of serology results. Colonization the upper respiratory passages only in young mice and rats may not stimulate suf detectable antibody. Where *M. pulmonis* infection is endemic, titers usually incre with age. Cross reactions with other murine mycoplasmas (e.g. *M. arthritidis*) ma encountered with the ELISA.

c. Interpretation: Cesarean-derivation and barrier-maintenance is the accepted method of producing *M. pulmonis*-free mice and rats. Failure to detect uterine infection with *M. pulmonis* at the time of the hysterectomy is the most logical explanation for introduction of the agent into the production colony. When *M. pulmonis* is introduced in this manner, good husbandry practices and the absence of infectious processes with other pathogens may limit infection to the upper respiratory tract, and horizontal spread may be limited. Under such conditions, serologic monitoring, even with very sensitive techniques such as the ELISA, of weanling mice and rats could fail to detect infection. Serologic monitoring of older mice and rats (exbreeders) is preferred. A combination of attempted isolation from several sites and serology contributes to confidence in the final determination.

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Pasteurella pneumotropica

Cynthia Besch-Williford and Joseph E. Wagner

1. **Agent:** ***Pasteurella pneumotropica*** **(Kingdom: Procaryotae; Division: Gracilicutes; Class: Scotobacteria; Order: not known; Family: Pasteurellacae; Genus:** ***Pasteurella*****). Disease induced: Pasteurellosis.**

   a. Morphology: *Pasteurella pneumotropica* is a small nonmotile coccobacillus.

   b. Cultivation: The organism produces 1-2 mm grey, smooth nonhemolytic or alpha hemolytic colonies on blood agar in aerobic atmosphere at 37°C by 24 to 48 hours. Most isolate will not grow on MacConkey agar and, if they do, it is usually after 48 hours. Biochemically, the most constant differential reactions include oxidase, catalase, urease, and nitrate reduction; glucose, maltose, and sucrose fermentation (acid withou gas); and lack of citrate utilization and of mannitol and sorbitol fermentation (table 1 Biochemical variants occur frequently and tend to revolve around the ability to produce indole.

   c. Strains: Serologic tests to evaluate the antigenic relatedness of *P. pneumotropica* isolates have not been widely used. However, three biotypes have been delineated.

2. **Characteristics of Infection and Disease**

   a. Hosts: *Pasteurella pneumotropica* has been isolated from a wide variety of laboratory anima species including rats, mice, kangaroo rats, guinea pigs, hamsters, and rabbits. There are reports of recovery of the organism from wild rats, poultry, dogs, and man.

   b. Geographic distribution: *Pasteurella pneumotropica* has a widespread distribution in conventional and sometimes barrier colonies of rodents in the United States, Europe, Asia, and Japan.

   c. Means of spread: Natural infections and even some experimentally induced infections with *P. pneumotropica* are clinically silent and may be considered latent with the bacteria readily isolated from the upper respiratory tract (nasopharynx, conjunctiva, nasolacrimal duct), trachea, lungs, and uterus. Other viscera are rarely colonized. *P. pneumotropica* can colonize and be maintained in the intestinal tract of axenic rats. I a colony of F344 rats affected with necrotizing mastitis caused by *P. pneumotropica*, the organism was also routinely isolated from fecal pellets. Overall, little has been do to investigate the occurrence of the organism in the feces of conventional rats or mice Uterine colonization has been hypothesized to occur following primary bacteremia or from an ascending infection as a result of genital grooming. It has been speculated th congenital infections occur when full-term fetuses are delivered through an infected reproductive tract. Other reports document nasopharyngeal colonization in rodents ju prior to or at weaning. Transmission is thought to occur by direct contact with secretions from the upper respiratory tract, uterus, or perhaps feces.

   d. Morbidity and mortality: *Pasteurella pneumotropica* has been incriminated infrequently as a primary pathogen respiratory, uterine, and subcutaneous infections of mice and rats; certainly the latenc of infection in both naturally occurring and experimental infections points to the role of the organism as a secondary opportunist of low pathogenicity that is triggered to a as a pathogen when immunologic barriers to disease are impaired by the genetic constitution of the species, or by the presence of a primary infectious pathogen or an immunosuppressive agent. Generally, morbidity and mortality data from latently infected rodent colonies are low.

   f. Prevalence: Early reports of microbial screening of conventional colonies of numerous mouse strains revealed a carrier incidence in the respiratory tract from 48 to 95 percent. Fro a number of surveys, uterine colonizations were reported to occur in 4 to 33 percent breeder and virgin mice raised in conventional facilities. Similar colonization rates ha

been implied for conventional rat colonies. Isolation of *P. pneumotropica* from barrier-maintained, gnotobiotic rodent colonies is less frequent. Infections in such colonies tend to be clinically silent.

f. Pathology: Disease caused by *P. pneumotropica* does not produce characteristic lesions of the infection. In clinically ill mice, the body systems most often affected include the upper and lower respiratory tracts and the reproductive tract; in the rat, disease associated with infection includes the upper and lower respiratory tracts and superficial tissues (i.e., skin and mammary gland). Upper respiratory tract lesions include mucopurulent conjunctivitis, hypopyon or panophthalmitis, sinusitis, and otitis media. Pulmonary infections include suppurative bronchopneumonia, frequently in the presence of other respiratory pathogens such as *Mycoplasma pulmonis* or Sendai virus. In reproductive tract infections where poor fecundity or abortions occurred, a necrotizing or suppurative metritis, and bulbourethral gland abscesses have been described. Pyogenic infections of superficial tissues are less common and include furunculosis, acute necrotizing mastitis and masseter muscle abscesses.

g. Diagnosis: The key test to diagnose *P. pneumotropica* infections is isolation of the organism from lesions or respiratory or genital mucous membranes. Isolation of *P. pneumotropica* from feces is difficult not only because of delayed growth or lack of growth on selective media such as MacConkey's agar, but also because of the presence of heavy fermenting bacteria such as *E. coli* or *Enterobacter* sp. which could potentially starve or overgrow the pinpoint colonies of *P. pneumontropica.* Immunofluorescent techniques have been developed to identify bacterial antigens in infected tissues. Use of agglutination or complement fixation techniques for identification of antibody titers to *P. pneumotropica* were found to be impractical since titers were found only in animals with overt disease and not in carrier animals.

h. Interference with research: Little information is available on the specific impact of overt or latent *P. pneumotropica* infections on research. The risk of triggering latent infections into overt disease, especially in naive or immunologically incompetent animals, warrant acquisition of *P. pneumotropica*-free rodents for research projects.

3. Control/Prevention

a. Zoonosis: *Pasteurella pneumotropica* infections in humans are rare. There are several reports of local *P. pneumontropica* sepsis resulting from bite wounds inflicted by dogs or cats. In only one instance was *P. pneumotropica* reported to have been isolated from the biting dog's mouth. Zoonotic infections from diseased rodents have not been reported.

b. Effectiveness of antibiotic treatment: In most colony situations, treatment is unfeasible. In a breeding colony of nude mice where the value of the animal was great enough to warrant treatment, antibiotic regimens of ampicillin and oxytetracycline produced regression of clinical signs. Ampicillin effectively cleared the mice of a carrier state (as determined by nasopharyngeal cultures); oxytetracycline did not. Another report indicated that chloramphenicol was effective in abating clinical signs of *P. pneumotropica* infections.

c. Effectiveness of cesarean derivation and barrier maintenance: *Pasteurella pneumotropica* may be excluded from rodent colonies by gnotobiotic derivation if the uteri are free from infection. Since infected uteri may carry fetuses to full term, failure to culture uterine mucosa at the time of cesarean derivation may result in introduction or reintroduction of the organism into isolator or barrier colonies.

4. Test Procedures

a. Isolation: *Pasteurella pneumotropica* can be readily isolated from the upper respiratory tract, trachea, lungs or uterus of affected animals. An aseptic culture technique should be used, followed by direct inoculation onto a 5 percent blood-agar plate.

Table 1.—Characteristic biochemical reactions of *Pasteurella pneumotropica*

Characteristic	*P. pneumotropica*
Catalase	(+)
Oxidase	(+)
Growth on MacConkey	(−)
Carbohydrate, acid from:	
Glucose	(+)
Sucrose	(+)
Lactose	(+)
Maltose	(+)
Mannitol	(−)
Sorbitol	(−)
Xylose	(+)
Salicin	(−)
Raffinose	(+)
Citrate utilization	(−)
Nitrate reduction	(+)
Nitrite reduction	(−)
Indole	(+)
Gelatin hydrolysis	(−)
Urease	(+)
Methyl Red/Voges-Proskauer (MR/VP)	(−/−)

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Actinobacillus spp.

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1. Agent: *Actinobacillus* spp. including *Actinobacillus lignieresii* and *Actinobacillus equuli* (Kingdom: Prokaryotae; Division: Gracilicutes; Class: Scotobacteria; Order: not known; Family: Pasteurellaceae; Genus: *Actinobacillus*).

a. Morphology: Actinobacilli are small pleomorphic, Gram-negative coccobacilli that produce 1-2 mm, raised, smooth, grey-white, nonhemolytic colonies on blood agar in 24 hours under aerobic conditions.

b. Cultivation: Reactions expected of *Actinobacillus* spp. include oxidase production and rapid urea hydrolysis, negative methyl red, Voges-Proskauer and indole (Ehrlich's) tests and acid-over-acid reaction on TSI. Nitrate reduction and glucose fermentation occur within 24 hours. They grow on MacConkey agar and are beta-galactosidase positive (o-nitrophenyl-beta-D-galactopyranoside test). Table 1 summarizes the fermentative patterns of *Actinobacillus equuli* and *A. lignieresii*, the two most commonly isolated actinobacilli in rodents. *Actinobacillus* spp. are biochemically similar to *Pasteurella pneumotropica* and *Pasteurella urea* and can be differentiated based on a complement of tests: urease and beta-galactosidase activity, lack of indole production and growth on MacConkey agar. Although some *P. pneumotropica* and *P. urea* isolates share one or more of these biochemical characteristics, none respond identically to all four tests. However, differentiation of slightly atypical cultures is often elusive and based on sample origin under conditions of practical laboratory diagnosis.

c. Strains: No studies have been performed to evaluate the physiologic and antigenic relatedness of *Actinobacillus* spp. isolated from rodents with those obtained from domestic livestock, poultry, or man. Based on culture characteristics alone, eight isolates of *A. lignieresii* of human and bovine origin were similar, differing in fermentation of mannose and arabinose and production of catalase and H_2S. Using gel precipitation, various degrees of serologic cross-reaction were demonstrated among the bovine and human isolates. An extensive study characterizing multiple avian isolates of *Actinobacillus* and *Pasteurella* spp. and comparing their characters with related species of *Actinobacillus* and *Pasteurella* revealed the confusion of extact identification which seemed dependent on origination of isolant, commercial source of media or substrate, length of incubation, and the uncertain guidelines for speciation within the genus *Actinobacillus*.

2. Characteristics of Infection and Disease

a. Hosts: *Actinobacillus* spp. have been recovered from both healthy and diseased rodents including mice, rats, hamsters, and guinea pigs.

b. Geographic distribution: *Actinobacillus* spp. infections have been reported to occur in conventional rodent colonies worldwide. However, due to the limited recognition of the bacteria as a potential pathogen, the identification of infection in conventional as well as barrier colonies may not yet be fully realized.

c. Means of spread: At present, there is no published information establishing the mode of transmission. Several reports indicate isolation of *Actinobacillus* spp. from the posterior nasopharynx and feces of asymptomatic conventional rodents, suggesting that the bacteria may be spread by direct contact with feces or oral secretions. *Actinobacillus* spp. have also been recovered from cultures of tap water; this tends to imply a saprophytic rather than a commensal role in colonization of the rodent gut.

d. Morbidity and mortality: Due to limited recognition of *Actinobacillus* spp. as potential pathogens, there are no existing reports establishing morbidity and mortality data. The organisms appear to be commensal and possibly saprophytic in nature, and can be cultured from asymptomatic, apparently healthy animals.

e. Prevalence: Prevalence data on the recovery of *Actinobacillus* spp. from laboratory animal colonies have not been established.

f. Pathobiology: Naturally occurring disease states from which *Actinobacillus* spp. were isolated include otitis media in rats and mice, conjunctivitis in a guinea pig, and abscessation of a hamster cheek pouch. Earlier reports have indicated that experimental inoculation of white mice with *A. equuli* or *A. lignieresii* failed to produce lesions or death. Experimental parenteral inoculations with *Actinobacillus equuli* and *A. lignieresii* revealed no morbidity or mortality in selected inbred and outbred rats or mice with the bacteria routinely isolated from the posterior nasopharynx, uterus and feces. No vertical transmission was documented. In rabbits and guinea pigs, there is reported to be variable virulence with *A. lignieresii* inoculations; subcutaneous injections induced abscess formations with concurrent liver and spleen granulomata and occasionally death. Intravenous and intraperitoneal injections resulted in high mortality with visceral microgranulomata and fibrinopurulent peritonitis respectively.

g. Diagnosis: The primary isolation of *Actinobacillus* strains can be accomplished from culturing nasopharynx, feces, or exudate from a lesion onto blood agar plates. Isolates that produce small, grey, nonhemolytic colonies, stain Gram negatively and are oxidase positive should be inoculated into a battery of media including TSI, urea, MacConkey, dextrose (with Andrade's indicator) and nitrate agars to evaluate biochemical activity consistent with *Actinobacillus* spp. Fermentative activity can then be evaluated for definitive speciation.

h. Interference with research: It is not known what degree of impact, if any, occurs in research protocols using asymptomatic, apparently healthy rodents carrying *Actinobacillus* spp. It appears that a decline in the immune competence of the animal predisposes to overt actinobacillosis such that the presence of the bacteria may alert to the presence of other stressors that may affect the quality of research data collected.

3. Control/Prevention

a. Special considerations: *Actinobacillus* spp., especially *A. lignieresii*, are considered parasites, entering wounds and causing granulomatous reactions. There are frequent reports of granulomatous disease in cattle and lymphadenitis and septicemia in horses, swine, poultry, and man caused by *Actinobacillus* species. In all cases of human actinobacillosis, immunologic stressors such as diabetes or pregnancy were incriminated in predisposing to the bacterial infection. It is unlikely that animal-to-man transmission is incriminated in human infections because of the commensal role of the bacteria in nature.

b. Control: Due to the commensal and possibly saprophytic nature of the bacteria, the production and maintenance of rodent species in barrier conditions may prevent colonization of the alimentary tract. Since *Actinobacillus* spp. have been isolated from tap water and from domestic livestock including poultry, exposure of nonbarrier-maintained rodents may be prevented by providing distilled water for drinking and restriction of animal care personnel to rodent rooms if livestock species are housed in the same facility.

4. Test Procedures

a. Isolation: Isolation of *Actinobacillus* spp. from the nasopharynx, feces, or suspect lesions can be accomplished by utilizing an aseptic culture technique followed by direct inoculation onto 5 percent blood agar and MacConkey's agar plates. Care must be taken to differentiate *Actinobacillus* spp. isolates from *Pasteurella* isolates (see **"Cultivation"**).

Table 1 - Fermentative reactions of *A. lignieresii* and *A. equuli*

	Fermentative Reaction[a]			
	A. lignieresii		*A. equuli*	
	24 h	48 h	24 h	48 h
Carbohydrate	Clinical Isolates[b]	Clinical Isolates	Clinical Isolates[c]	Clinical Isolates
Glucose	+	+	+	+
Xylose	+	+	+	+
Mannitol	+(−)	+ (−)	W	+
Lactose	−	−	−	+
Sucrose	+	+	+	+
Maltose	+	+	+	+
Trehalose	−	−	+	+
Salicin	−	W	−	−
Arabinose	+	+	+ (I)	+

[a] + = Positive reaction (fermentation); − = Negative reaction (no fermentation); (−) = One strain was negative; W = Weak acid production; I = Some strains were negative at 24 hours.

[b] The two clinical *A. lignieresii* isolates.

[c] The clinical isolates of *A equuli*.

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Salmonella spp

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1. **Agent: *Salmonella* spp. (Kingdom: Procayotae; Division: Gracilicutes; Class: Scotobacteria; Order: not known; Family: Enterobacteriaceae; Genus: *Salmonella*). Disease induced: Salmonellosis.**

   a. Morphology: Small Gram-negative rods measuring 2-4 μm by 0.5-0.6 μm, usually motile with peritrichous flagella.

   b. Cultivation: Selective media containing chemicals such as brilliant green, crystal violet, bile salts, and tetrathionate can be used for isolation. Citrate can be used as the carbon source. Glucose is fermented with the production of acid and gas. There is rare fermentation of lactose and sucrose. Indole is formed in rare cases. They actively produce H_2S and reduce nitrate and decarboxylate lysine.

   c. Strains: Type species: *Salmonella cholerae-suis*. *Salmonellae* are classified into many serovars according to differences in O, Vi and H antigens (Kaufmann and White scheme). Phage-typing is also performed. Salmonellosis in mice is caused mainly by *S. typhimurium* and *S. enteritidis*. *S. newport, S. cerro, S. infantis, S. montevideo, S. livingstone, S. agona,* and *S. oranienburg* also have been reported to cause mouse salmonellosis.

   d. Stability: *Salmonellae* are relatively stable in dry environments, and strains inoculated in media can survive for more than 1 year at ordinary room temperature (23 °C).

   e. Inactivation: Most *Salmonella* strains can be killed by exposure to 55 °C for 1 hour or 60 °C for 20 minutes. Many kinds of disinfectants including sodium hypochlorite (1,000 ppm of active chloride), iodophores (150-300 ppm) and formaldehyde are effective.

2. **Characteristics of infection**

   a. Animals: *Salmonella* infections occur in many species of animals. Mice and guinea pigs are more susceptible to the bacteria than other species of animals. Clinical symptoms in mice are severe in BALB/c and C57BL/6. A/J, C3H/He and CBA are relatively resistant.

   Humans: *Salmonellae* are important zoonotic agents. They can cause food poisoning and diarrhea in man.

   b. Geographic distribution: Salmonellosis is a pandemic disease. The bacteria are widely distributed in many species of animals and man.

   c. Means of spread: *Salmonellae* are transmitted by the oral route. The conjunctiva is a possible route of infection. Within a colony, contact infection is the most important means of spread of disease. Contaminated diets and bedding may be sources of infection if there are species of latently infected animals in the animal facility environment.

   d. Morbidity and mortality: Morbidity may vary from a few percent to more than 75 percent depending on factors such as the species of *Salmonella* and the strain and stock of mice. Mortality is influenced by the same factors that affect morbidity. Age and experimental manipulation may also affect mortality.

   e. Prevalence: Salmonellosis was an important disease in mouse colonies in the past, with a prevalence approaching 50 percent. Recently, the disease is rarely detected in mouse colonies because of elimination of latent infections and hygienic management.

   f. Pathology: Mice may die of acute septicemia with few obvious lesions. In subacute or chronic cases, mice may develop diarrhea, anorexia, and loss of weight. They usually show characteristic lesions including enlargement of the spleen and liver, often associated with varying degrees of greyish-white protuberant necrotic foci, the so-called "typhoid nodules."

g. Diagnosis: Isolation of *Salmonella* from blood and/or visceral organs is indispensable for diagnosis of acute salmonellosis. Gross pathology is valuable for diagnosis of subacute or chronic salmonellosis, but confirmation by isolation of the causative agent is necessary. Serology is subsidiary to cultivation for diagnosis of salmonellosis.

h. Interference with research: Salmonellosis is potentially a major cause of interference with research. Colonies of susceptible mice may be decimated on first contact with highly virulent species. Stress such as shipping and experimental manipulation may provoke latent infection.

3. Control/Prevention

a. Special considerations: Eradication of *Salmonella* should be started by elimination of affected colonies and disinfection of contaminated rooms and equipment. Infected laboratory rodents and guinea pigs, as well as feral rodents, birds, and cockroaches are important sources of the infection. Detection and elimination of inapparently infected animals and sterilization or pasteurization of bedding and diets are effective for prevention of recontamination.

b. Effectiveness of vaccination and antibiotic treatment: Vaccination cannot prevent latent infections but protects mice against lethal challenge doses. The use of antibiotics, including chloramphenicol and oxytetracycline, can reduce mortality in epizootics of salmonellosis but cannot eliminate the carrier state in chronically infected animals.

c. Effectiveness of hysterectomy derivation: Hysterectomy is effective for rederivation of contaminated mouse stocks.

4. Test procedures

a. Isolation: Isolation of the organism is required to confirm the diagnosis of salmonellosis. Cecal contents, feces, visceral lesions, and lymph nodes are suitable specimens for isolation of *Salmonella*. Selective enrichment and isolation of *Salmonella* can be accomplished using such media as Gram-negative broth or Rappaport broth, and brilliant green agar, MacConkey agar, or DHL (desoxycholate-hydrogen sulfide-lactose) agar.

b. Serology: Serological tests are subsidiary to isolation for detection of latent infection. The agglutination test using heat-killed antigen can be employed.

c. Histological examination: Necrotic lesions in the spleen and liver may be found in subacute and chronic infections. Isolation of *Salmonella* is necessary for differential diagnosis from Tyzzer's disease, corynebacteriosis, and mousepox.

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Streptococcus pneumoniae

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1. **Agent:** ***Streptococcus pneumoniae*** **(Kingdom: Procaryotae; Division: Firmicutes; Class: Firmibacteria; Order: not known; Family: Streptococcaceae; Genus:** ***Streptococcus*****). Disease induced: Streptococcosis.**

   a. Morphology and special character: Gram-positive, capsulated, spherical cocci occurring mostly in pairs, but sometimes in short, straight chains. Nonmotile. Identification is made by positive reactions in optochin susceptibility test, in addition to morphological and cultural characteristics. They are easily soluble in bile, but this character is more variable than optochin sensitivity.

   b. Cultivation: Good growth is obtained on blood agar and in trypticase soy broth. At 24 hours, tiny, smooth, transparent, glistening, flat colonies surrounded by narrow zones of α-hemolysis are seen on blood agar. At 48 hours, colonies show concentric ring appearance. Diffuse growth occurs in trypticase soy broth and in serum broth. They are facultative anaerobes, but some strains are carbon dioxide dependent. Anaerobic incubation usually enhances capsular formation and growth and produces a more typical colony. Optimum temperature is 37°C.

   c. Strains: Eighty-three different serotypes (48 groups and 83 types) are shown by the capsular reaction test (Neufeld test or quellung reaction). Serotypes of a given strain are first examined in pooled polyvalent sera and then in relevant type or group sera. There are some serological cross-reactions between pneumococci and capsulated nonhemolytic streptococci, *Escherichia coli*, *Klebsiella* , *Salmonella kirkee* and *Haemophilus influenzae.*

   d. Virulence: Virulence of *S. pneumoniae* is entirely correlated with its capsular material. Thus smooth encapsulated organisms play the key role in pathogenesis, namely by resisting defense mechanisms, surviving, and subsequently multiplying in host tissues. Animals may be infected with rough and/or smooth forms but disease is caused strictly by the latter.

   e. Stability: Pneumococci readily autolyze. The number of viable organisms in trypticase soy broth culture rapidly decreases after 20 hours. They are killed by heat at 60°C for 30 minutes, are labile at -20°C and stable at temperatures lower than -40°C.

   f. Inactivation: Pneumococci are easily killed with ordinary disinfectants for bacteria.

2. **Characteristics of infection and disease**

   a. Animals: Guinea pigs are highly susceptible to infection by pneumococcus type 19, suffering from pneumonia, pericarditis, pleuritis, peritonitis, meningitis, and septicemia. These lesions caused by pneumococcus types 2, 3, 8, 16, and 19 have also been reported in rats. Pneumococcal septicemia and mastitis are found in calves, cattle, sheep, and goats, while pneumococcal infection in monkeys is usually restricted to the respiratory tract, producing lobar pneumonia. Pneumococci are virulent in mice, but natural infections are rarely found.

   b. Humans: Various types of *S. pneumoniae* have been found as causative agents of lobar pneumonia, bacteremia, purulent meningitis, pericarditis, arthritis, abscesses, and infections of the middle ear, sinuses, eyes, and skin. Although isolations of types 1, 2, 3, 6, 7, 9, 14, 18, 19, and 23 have been relatively frequent in humans, predominant types have changed at different times in different areas or countries.

   c. Geographic distribution: Pneumococcal infections of laboratory rodents and monkeys seem to be distributed worldwide.

d. Means of spread: The organism is transmitted among animals by contact with infected animals and animal handlers carrying the organism. Natural infections occur through the intranasal route.

e. Morbidity and mortality: Most animals infected with *S. pneumoniae* are asymptomatic carriers which harbor the organism usually in the upper respiratory tract. Morbidity and mortality are indefinite, because onset of clinical signs and lesions are accelerated by predisposing factors such as stress of pregnancy, shipment, dietary deficiencies, concurrent infections and experimental procedures. In early descriptions of this infection in guinea pigs, morbidity and mortality were probably influenced markedly by vitamin C deficiency, general poor nutrition, and inappetence.

f. Prevalence: Prevalence of the infection is influenced by the predisposing factors described above, and therefore is highly variable. In recent times, *S. pneumoniae* is rarely found in rat breeding colonies, but sometimes detected in guinea pig colonies. According to some investigators, carrier rates in guinea pig colonies varied from 15 to 55 percent. Transmission of the organism is not always successful even in cagemates with infected animals. It is generally believed that about 40 to 70 percent of healthy people are carriers of pneumococci.

g. Pathology: Fibrinous lobar pneumonia, pericarditis, and pleuritis are commonly found in pneumococcal infections in guinea pigs and rats. Less commonly, fibrinous peritonitis, otitis media, suppurative meningitis, and septicemia are observed. The major histopathological findings are fibrinopurulent pneumonia or pleuropneumonia in which congestion of the alveolar capillaries and diffuse infiltration of polymorphonuclear cells and serofibrinous exudate in the alveolar spaces are observed. The pleura is remarkedly thickened with fibrin, serum and inflammatory cells. Histological features of the pericarditis and peritonitis are similar to those of the pleuritis. Pneumococci are found abundantly in the inflammatory exudate and inside the polymorphonuclear cells. In monkeys, lobar pneumonia is the major lesion.

h. Diagnosis: Diagnosis is made by isolation of the organism from affected tissues, upper respiratory tract, and throat. Saline washings of the tympanic cavity may be used for this purpose in rats. Suspicious colonies grown on blood agar are identified by morphological and cultural characteristics and optochin sensitivity. Serological typing of the organism is performed by the quellung reaction and agglutination test with specific antisera. Although ELISA seems to be useful for serological checking in rats, no method has been established for routine serological diagnosis.

i. Interference with research: Animals showing clinical signs cannot be used for research. Inapparent infections may be provoked into clinical illness by certain kinds of experimental procedures.

3. Control/prevention

a. Special considerations: *S. pneumoniae* infection is regarded as zoonotic because identical serotypes of pneumococci are found in man and animals and, in addition, there have been reports of apparent natural transmission of pneumococci between animal handlers and calves.

b. Effectiveness of antibiotic treatment: *S. pneumoniae* is sensitive to penicillin, chloramphenicol, and sulphonamides. However, penicillin-resistant strains may be isolated from natural infections. Antibiotic sensitivity tests should be performed prior to treatment. Although antibiotics are useful in the cure and prevention of systemic infections such as septicemia, pneumonia, and pleuritis, they are usually regarded as ineffective in elimination of the organism from the upper respiratory tract. Treatment of guinea pigs with antibiotics should be done with care since fatal enteritis may result. Pneumococcal vaccines consisting of capsular polysaccharides are available for human use, but early limited studies with vaccines in animals have failed to demonstrate a beneficial effect.

c. Effectiveness of hysterectomy derivation: Pneumococcal infection is never seen in hysterectomy-derived animals that are protected from contamination. However, careful operation and checking should be done, because pneumococci are isolated occasionally from the vagina.

4. Test procedures

a. Isolation: Swabs of the upper respiratory tract and cut surface of lesions are inoculated on blood agar plates and incubated both aerobically and anerobically at 37°C for 24 to 48 hours. Pneumococcal colonies are sometimes overgrown by fungi and other species of bacteria after prolonged incubation. A suspicious colony is rather densely inoculated on a blood agar plate and an optochin disk is placed on it. After overnight incubation at 37°C, a growth inhibitory zone of 5 to 30 mm is observed. About 80 to 90 percent of carriers can be detected with nasal swabs from live animals, but usually only a few colonies are yielded from these samples. Therefore, repeated samplings are recommended. There are no selective media for *S. pneumoniae*.

b. Serology: Antibody response is recognized in pneumococcal infections in rats, but serology is not used routinely for diagnosis.

c. Interpretation: Although occasional viridans streptococci are positive in the optochin test, the test is reliable for identification of pneumococcus when reference is made to morphological and cultural characteristics. Bile solubility and virulence in mice are supplemental characters useful in identification of *S. pneumoniae*.

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III. Parasites

Spironucleus muris

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1. **Agent:** ***Spironucleus (Hexamita) muris*** **(Phylum: Protozoa; Subphylum: Sarcomastigophora; Class: Zoomastigophora; Order: Diplomonadida; Genus:** ***Spironucleus*****). Disease induced: Spironucleosis.**

   a. Morphology: *Spironucleus muris* is a flagellated protozoon that measures 7 to 10 μm long and 2 to 3 μm wide. The trophozoite is elongated, piriform shaped, and bilaterally symmetrical. It has four pairs of flagella arising from a pair of blepharoplasts located between two anterior nuclei, as well as two longitudinal axostyles.

   b. Life cycle: *S. muris* multiples by longitudinal fission. Trophozoites are commonly found in the crypts of Lieberkuhn in the anterior small intestine. Infectious, resistant cysts are passed in the feces. The prepatent period after ingestion of the cysts can be 2 to 3 days if the infective dose is high, and up to 6 days if the infective dose is low.

2. **Characteristics of infection**

   a. Hosts: *Spironucleus muris* is a facultative pathogen of laboratory mice, rats, hamsters, and wild rodents. It is not known to infect man.

   b. Geographic distribution: Worldwide.

   c. Means of spread: Transmission is through ingestion of resistant infectious cysts. The cysts are shed with the feces in waves, and young or immunosuppressed animals seem to shed greater numbers. Within enzootically infected colonies, sucklings are probably infected in the parental cage and may develop clinical disease following periods of stress such as weaning.

   d. Predisposing factors: *Spironucleus muris* is more pathogenic for young (weanling), stressed (crowding, cold, diet changes, malnutrition, etc.), immunosuppressed (athymic, irradiated, etc.) or inbred animals. Mice seem to be more susceptible than other rodent species, and there appears to be a mouse strain difference in susceptibility.

   e. Morbidity and mortality: Spironucleosis can be an acute or chronic disease of laboratory animals. High mortality and morbidity in inbred, weanling, immunosuppressed, or stressed mice has been reported by several authors.

   f. Prevalence: The prevalence of *S. muris* in enzootically infected colonies is variable, depending on predisposing factors such as those discussed above, as well as colony management procedures.

   g. Pathobiology: Infected animals may exhibit nonspecific signs such as depression, rough hair coat, mild diarrhea, dehydration, weight loss, listlessness, hunched posture, and a distended abdomen. High postweaning mortality and sporadic deaths have been reported in affected mouse colonies. The parasites are often found in mouse, rat, and hamster colonies where few if any clinical signs of disease are encountered. Necropsy of affected animals may reveal an accumulation of colorless to gray-brown catarrhal fluid in the anterior small intestine.

   Histophatological lesions are not a consistent feature of the disease; however, histopathologic lesions may include duodenitis accompanied by accumulations of catarrhal fluid and spironucleus organisms in the crypts as well as the lumen between villi. Degeneration of the villar epithelium and organismal invasion of the mucosa and lamina propria may be seen. In chronically affected mice, there may be hyperplasia and increased mitotic activity of the intestinal epithelium. The crypts may be distended or cyst-like and filled with inflammatory and cellular debris, mucoid material, and organisms which contain a double nucleus at one end.

h. Diagnosis: Diagnosis of *Spironucleus muris* is based on the demonstration of trophozoites in the intestinal lumen or crypts of Lieberkuhn in the small intestine. Microscopic examination of multiple H&E sections of the anterior to middle third of the small intestine and pylorus (rats) usually reveals the parasite. Stained direct smear preparations or wet mounts examined by phase-contrast microscopy will reveal *Spironucleus* organisms.

Demonstration of cysts in the feces of affected animals is also diagnostic of spironucleosis but may be unreliable because of intermittent shedding or declining numbers as the animals get older. The immature and mature stages of the cysts appear banded, with an "Easter-egg" appearance upon Giemsa staining. Unstained, mature cysts from the feces are ovoid, appear dark, and measure around 4 to 7 μm in diameter.

i. Interference with research: In addition to causing morbidity and in some cases mortality among infected research animals, a few of the many ways that infection with *Spironucleus muris* may interfere with research include:

1. Alterations in macrophage activity and metabolism.
2. Interference with the host immune response.
3. Increased susceptibility to whole body irradiation.
4. Increased mortality in cadmium exposed mice.
5. Conversion of inapparent Sendai infection into overt clinical disease.
6. Shortening of the life span of athymic mice.
7. The presence of activated macrophages in the host which kill tumor cells nonspecifically in in vitro assays.
8. Poor response to a wide variety of antigens in comparison with uninfected animals, making infected animals unsuitable for immunological studies.

3. Control/Prevention

a. Prevention and control: This is based on the establishment of proper sanitation and good colony management. Cross-species transmission of *Spironucleus* among mice, rats, and hamsters may occur.

The infectious cysts are sensitive to high temperatures (+45°C) and most common disinfectants, but are resistant to dessication, freezing, and low pH.

b. Treatment: Treatment of mice with clinical disease has been accomplished with 0.04-0.1 percent dimetridazole (Emtryl® Soluble Powder, Salsbury Labs, Charles City, Iowa) in drinking water for 14 days, but this did not eliminate parasites from infected animals. Infected colonies remain infected until rederived.

c. Effectiveness of hysterectomy derivation: The establishment of cesarean-derived colonies eliminates infections. Effective barrier housing methods are needed to maintain a *Spironucleus*-free status. However, *S. muris* has been reported as a frequent cause of the breakdown of barrier-sustained breeding units in England.

4. Test Procedures

a. Histopathology: Microscopic examination of multiple H&E sections of the anterior to middle third of the small intestine may reveal trophozoites in the lumen or crypts of Lieberkuhn.

b. Necropsy: Intestinal content smears or wet mounts examined by phase-contrast microscopy may reveal *Spironucleus muris* organisms. The trophozoites can be distinguished from other protozoa by their small size, lack of undulating membrane, and quick horizontal or zigzag movements.

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Syphacia spp.

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1. **Agent:** ***Syphacia obvelata*** **(mouse pinworm)** ***Syphacia muris*** **(rat pinworm) (Phylum: Aschelminthes; Class: Nematoda; Subclass: Secernentea; Order: Ascarida; Suborder: Oxyurina; Superfamily: Syphacioidea; Genus:** ***Syphacia*****). Disease induced: Oxyuriasis.**

   a. Morphology: Small to medium roundworms, with three distinct lips, small cervical alae, no buccal capsule, possessing a prominent esophagus with a well-developed single bulb at the posterior end. Adults are cylindrical with a rounded anterior region and a long, pointed tail. Male worms possess three ventral cuticular projections (mamelons), a single, long prominent spicule and a gubernaculum, and the tail is bent ventrally. The tail of the female is long and pointed, with the vulva located on the anterior quarter of the body, behind the excretory pore. The eggs are asymmetrical, thin-shelled, and therefore rather transparent.

   b. Species differentiation: *S. muris* closely resembles *S. obvelata*, and can be differentiated from *S. obvelata* by the following criteria:

   1. The position of the excretory pore in both sexes and the vulva of the female is further posterior in relation to the esophageal bulb in *S. muris* than *S. obvelata.*
   2. The tail of the male *S. muris* appears thinner than that of *S. obvelata.* The tail length is about two times the body width, whereas the tail length of *S. obvelata* is about equal to body width.
   3. In *S. muris*, the anterior mamelon of the male is close to the middle of the body, while in *S. obvelata* the middle mamelon is close to the middle of the body.
   4. The eggs of *S. muris* are slightly more than half the length of *S. obvelata* eggs, although nearly the same width. *Syphacia muris* eggs are slightly asymmetrical, being flatter on one side than the other, while *Syphacia obvelata* eggs are banana shaped. Eggs of *S. obvelata* measure 111 to 153 by 33 to 55 microns, averaging 134 by 36 microns. Eggs of *S. muris* measure 72 to 82 by 25 to 36 microns, averaging 75 by 29 microns.
   5. *Syphacia muris*: Adult male is 1.2 to 1.3 mm long, 100 microns wide. Adult female is 2.8 to 4.0 mm long, 250 microns wide.
      Syphacia obvelata: Adult male is 1.1 to 1.6 mm long, 110 to 140 microns wide. Adult female is 3.4 to 5.8 mm long, 240 to 400 microns wide.

   c. Life cycle: The life cycle of *Syphacia* is direct, with a prepatent period of 8 to 15 days. After ingestion of embryonated ova by the host and subsequent hatching, the larvae grow and molt, becoming sexually mature in 6 to 7 days. After fertilization, the male dies, and is passed with the feces. Gravid females migrate from the intestines to the rectum, deposit eggs on the perianal skin, and die soon thereafter. The eggs become infectious in a few hours.

2. **Characteristics of infection**

   a. Hosts: Pinworms are commensal, relatively nonpathogenic, ubiquitous, bacteria-feeding roundworms, which occur primarily in the cecum and colon of the host. *Syphacia muris* is commonly found in the laboratory rat and wild rat, and occasionally in laboratory mice. There have been reports of *S. muris* in hamsters and gerbils. *Syphacia obvelata* is the most common nematode of the mouse. It can occasionally be found in rats, hamsters, and gerbils. Cross-infections with both *S. muris* and *S. obvelata* can occur when naturally infected mice and rats are kept in the same room. Infections with the nonautochthonous species are generally light and short-lived. However, they may be self-perpetuating in enzootically infected breeding colonies. Neither *S. muris* nor *S. obvelata* is regarded as a parasite of man, although occasional natural infections have been reported.

b. Geographic distribution: *Syphacia obvelata* occurs in most conventional and some "barrier" mouse colonies worldwide. Because *S. muris* has been confused with *S. obvelata* for many years, its exact geographic distribution is unknown, but it appears to be present in most conventional rat colonies throughout the world.

c. Means of spread: Animals are infected by ingesting embryonated eggs taken directly from the perianal area of self or cage mates during mutual grooming activities. Eggs can also be ingested in feces or feces contaminated feed, water, or debris. Retroinfection may also occur.

d. Morbidity and mortality: Pinworms are highly infectious helminths. Within enzootically infected rodent colonies, a high percentage of animals will harbor the organisms. Pinworm parasites of laboratory rodents are generally nonpathogenic, and infections are usually symptomless.

e. Prevalence: Pinworm infection in both wild and laboratory rodents is common worldwide. *Syphacia* species infestation in mice has been reported to occur in conjunction with both *Aspicularis tetraptera* and *Hymenolepis nana* infestations.

f. Predisposing factors: Animals with diminished resistance are more susceptible to infection. The prevalence of pinworms may be a function of age, sex, strain, and host status in enzootically infected colonies, newly weaned animals develop the heaviest parasite load. Male hosts are more heavily parasitized than females, and *Syphacia* numbers diminish with age of the host.

g. Pathobiology: *Syphacia* occurs in the cecum and colon of infected animals. Although pinworm parasites of laboratory rodents are generally nonpathogenic, heavy pinworm infections have been reported to cause rectal prolapse, constipation from impaction, intussusception, and mucoid enteritis of the cecum and large intestine. Clinical signs of pinworm infection are rare, but poor condition, rough hair, decreased growth rate, occasional diarrhea, and increased stickiness of fecal pellets have been ascribed to *Syphacia* infections.

h. Immunity: Mice develop an age resistance to *S. obvelata* infection between the 5th and 7th week of age (Sasa et al., 1962). Strains of mice vary in their resistance to infection (Harkness and Wagner, 1983; King and Cosgrove, 1963).

i. Diagnosis: The diagnosis of *Syphacia* infection is based on the demonstration of eggs on the perianal region (the cellophane tape technique), or the demonstration of adult worms in the cecum and colon at necropsy.

j. Interference with research: While pinworms are minimally pathogenic, it is generally agreed that laboratory animals heavily infected with gastrointestinal parasites produce inferior biological products, make poor experimental animals, and in the end, may greatly increase research costs. Heavily parasitized animals are not suitable for critical work such as nutrition, immunology, or blood studies, because normal physiological reactions and blood counts cannot be obtained. Under many conditions, infection with a small number of intestinal parasites may produce no apparent clinical effects, but under experimental conditions, the lowered resistance of the animals may give rise to unreliable, nonrepeatable, or misleading results. Parasitic nematodes may also transmit, provoke, or enhance bacterial and viral diseases, and suppress or enhance tumor growth and humoral responses (Blair et al., 1968; Habermann and Williams, 1958; McNair and Timmons, 1977).

3. Control/Prevention

a. Treatment: Most drugs, such as piperazine compounds, are effective in eliminating adult worms, but are not efficient in clearing immature worms. Additionally, the possibilities for reinfection and infection by eggs from the environment must be considered. Thus, repeated treatment is necessary. The interval between subsequent treatments should be shorter than the prepatent period of the parasite to avoid further contamination of the

environment with newly hatched eggs. Three or four consecutive, weekly treatments are recommended. For maximum effectiveness, an entire room or facility must be retreated at one time. After treatment, animals should be moved to a clean area to prevent reinfection. One investigator went so far as to bathe and scrub the perianal areas of recently treated mice before moving them to a new, clean, egg-free environment. Some treatments that have been used with various degrees of success are the following:

Piperazine adipate—4 to 7 grams per liter of drinking water for 3 to 10 days.

Piperazine citrate—3 grams per liter of drinking water in a 3 week treatment (7 days on, 7 off, 7 on).

Pyrvinium pamoate—0.8 milligram per liter of drinking water or 1.2 to 1.6 milligrams per kilogram food over a 30 day period (mouse). Three milligrams per liter drinking water or 12 milligrams per kilogram in food for 30 days (rat).

Trichlorfon —1.75 grams per liter of drinking water for 14 days or 0.5 milligram per gram of food for 1 day (often combined with atropine and sucrose for palatability).

Dichlorvos—0.5 milligram per gram of food for a 24 hour period, repeated four times at 7 day intervals.

Thiabendazole —0.3 percent in food for 7-14 days.

Dithiazanine iodide —orally in food at 0.1 milligram per gram of food for 7 days.

Mebendazole—orally in food at 500 milligrams per kilogram as a single dose.

b. Control: Control is based on the use of adequate hygienic measures to remove parasitic eggs from the environment. Pinworms frequently appear in "barriers," suggesting that the eggs survive formaldehyde vapor decontamination efforts. Eggs have been found on equipment, in dust, and in ventilation ducts. A thorough cleaning and sterilization of cages and rooms is necessary. These hygienic measures should be combined with a program of effective drug therapy or cesarean derivation. Treatment should not be implemented without concern for the effects it may have on the research on which the animals are subsequently used.

c. Prevention: Prevention of pinworm infection in rodents is difficult and frequently ineffective. Pinworm infections can persist in many colonies despite what appears to be adequate control procedures. Pinworm-free animals can be readily reinfected if introduced into facilities containing parasitized animals. Dust and wild rodents should be reduced or eliminated, filter tops used, and cages, rooms, and air ducts cleaned frequently.

d. Effectiveness of hysterectomy derivation: Freedom from pinworm infection can most likely be achieved by establishing rodent colonies by cesarean derivation and through use of barrier housing methods. This technique is effective, but egg-contaminated dust and wild rodents can be a source of reinfection.

4. Test Procedures

a. Egg detection: The diagnosis of *Syphacia* infestation can best be accomplished by the cellophane tape technique. Clear cellophane tape is applied to the perianal region, and then examined for eggs on a glass slide under a microscope.

b. Necropsy: Adult worms can be identified in the cecum and large intestine at necropsy. The cecum and/or colon are opened and placed in a petri dish filled with warm water and allowed to settle for a few minutes. The organ contents are then examined with the naked eye or with a dissecting microscope for adult worms.

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